Report

on

FOURTH TECHNICAL ALFALFA CONFERENCE

American Dehydrators Association

and

Western Regional Research Laboratory
Agricultural Research Service, U.S.D.A.

July 26, 1957

Albany, California

PROGRAM

9:40 a.m.	General Situation and Program of A.D.A.	Mr. Joseph Chrisman American Dehydrators Assn. Kansas City 6, Missouri
10:00 a.m.	A.D.A. and Other Alfalfa Research	Dr. L. E. Card University of Illinois Urbana, Illinois
10:30 a.m.	Improving Alfalfa Through Research	Dr. H. O. Graumann Crops Research Division - ARS Beltsville, Maryland
11:00 a.m.	Biological and Chemical Control of Spotted Alfalfa Aphid	Mr. J. K. Holloway University of California Berkeley, California
11:30 a.m.	Upgrading Alfalfa by Mechanical Means	Mr. Joseph Chrisman
2:00 p.m.	The Influence of Stage of Maturity on the Utilization of Alfalfa	Dr. J. H. Meyer University of California Davis, California
2:30 p.m.	Comprehensive Analysis of Different Quality Dehydrated Forages	Dr. G. O. Kohler Western Regional Research Laboratory
3:00 p.m.	Occurrence and Stability of Vitamin E, K and Carotene in Alfalfa	Dr. P. A. Thornton Colorado State University Fort Collins, Colorado
3:30 p.m.	Occurrence and Nature of Estrogens in Green Forage Crops	Mr. E. M. Bickoff Western Regional Research Laboratory
4:00 p.m.	Review of Saponins in Forages	Dr. C. R. Thompson Western Regional Research Laboratory

GENERAL SITUATION AND PROGRAM OF A. D. A.

Joseph Chrisman

American Dehydrators Association
Kansas City, Missouri

The program of our American Dehydrators Association has many facets. In general, our aims are the same as those of any Trade Association; to promote the welfare of its members and the industry of which they are a part.

According to the objectives of the Association, as written into our Constitution by the founders, the welfare of the industry members can best be served through striving for greater uniformity of the customs and usages of those engaged in the dehydration of green forage crops, the inculcation of principles of justice and equity in business; the adjustment of business disputes; the dissemination of economic information, and the securing of the benefits of cooperative efforts through banding together. We have an additional aim, which is not common to all Trade Associations; the sponsoring of research, looking toward the improvement of products, better knowledge of their uses and limitations. In most industries this is a function of the individual firms.

The carrying out of these objectives is dependent on policy as expressed by our annual meeting of the membership and the directives of our Board and Executive Committees. Putting into execution these membership mandates and Board Directives is largely a function of the Executive Vice President and the Secretary of the Association. The various functions of our office must conform to, and be limited by, our ability to perform, and, more specifically, by our income. The Association has one major source of income--membership dues. Other minor incomes, such as charges for advertising in the Bulletin, and charges for reprints, are merely for the purpose of offsetting some of the costs of printing and mailing.

Our dues, as authorized at the most recent convention, amount to $125.00 per drum operated, to a maximum of eight units for any one member. Additional dues are collected on the basis of tonnage produced. By this method we collect .05¢ per ton of total amount up to a maximum of 100,000 tons per year. The dues collected under this latter phase are earmarked for two specific uses; research and promotion and advertising. This year, by authority of the convention, three-fifths of these tonnage collections are for our research program and two-fifths for promotion and advertising. We are also permitted to transfer 10 percent of tonnage dues collections to our operating fund for administration of the two specific programs, research and promotion. So much for the financial and of our business. It is most important, of course, because it both permits our activities and also limits their scope.

What are some of the things we actually do in Kansas City to carry out the objectives? We publish a weekly Bulletin in which, during the operating season, we attempt to give timely information to the members on weather and crop conditions in the various producing areas. This includes information on any serious insect outbreak, such as the spotted alfalfa aphid. Price information is a matter which must be handled with care. We try to use the latest published information, such as Agricultural Marketing Service's weekly price and also the prices announced to us through letters from some of the major blending firms. At intervals during the year we produce copies of charts showing week-by-week pricing of the product for several years, or annual production curves, hoping that members may profit by referring to what transpired in previous years with reference to the current situation. At the end of each month we make a survey of production and inventory for the current year and month and the past year and month and publish the results. Each month we also publish disappearance of alfalfa, as calculated from U.S.D.A. Market News Service figures, showing not only current disappearance, but monthly disappearance over the past five years. Occasionally we send the members tabulations, showing monthly prices for a five-year period for comparative use. Not every week, but rather frequently, we publish a Research News letter. We try to report in that letter late developments in alfalfa feeding trials, latest news on prevalence and manner of combating various alfalfa pests, new wrinkles in the agronomy of the plant, soil-treating practices or plant-breeding work. In issuing this Research News letter we are often assisted by the members of our Research Council. We prepare advertisements in our office for the feed trade magazines and livestock publications, and we provide reprints of many of these for members' use in selling the product and for inclusion in their Sales Kits. Mat service is provided to allow the industry members to place their own ads in their local papers. A number of magazine articles are written in our office, publicizing dehydrated alfalfa. We have enjoyed a monthly page in one of the feed trade magazines for several years; it is headed "Dehy Data". We have had our articles published in livestock papers, breed magazines and farm papers, and we have been instrumental in having special articles written by others for these various journals. Our Association provides group insurance for its members, permitting them to enjoy very low rates. Billing, premium notices, and collections are handled by the Kansas City office. Each year we hold either a Production School or a Sales Clinic for the exchange of ideas and looking toward product improvement and better selling methods. This year we are cooperating with the U. S. Public Health Service and Kansas Public Health Service in studying dust losses and their prevention. Our work here is principally liason between dehydrators in the field and the public health service people. We have joined with Agricultural Research Service and Monsanto Chemical Company in the effort to have Santoquin approved for use at non-toxic levels for all farm animals. We have joined forces with the Agricultural Research Service in other endeavors. Currently we are working toward hoped-for

modification of some of the rulings of the Wage and Hour and Public Contracts Division, U. S. Department of Labor. These rulings, we feel, are adverse to the interests of dehydrators and, in our opinion, unrealistic.

Turning now to the industry itself, we note that in the dozen years, from 1944-1956, it grew from 240,000 tons to about 1,200,000 tons, or 390 percent. During the same period, it is noted that the production of suncured alfalfa meal has declined from a half-million tons to less than 200,000 tons. It should be noted, however, that the increase in production of the dehydrated product has, by no means, been limited to the amount of drop in the naturally cured commodity. The total use of combined manufactured alfalfa products has shown an almost steady increase since the earliest days of dehydration.

In the fiscal year, just ended on April 30th, production was severely curtailed by widespread drought conditions and spotted alfalfa aphid depradations. Now, the drought seems well-broken and the aphid has been dormant in the midwest area. Nevertheless, production may still be curtailed and most certainly will not reach the previous peak this year. This is because of excessive moisture over a long period in some of the greatest producing areas of the middlewest. Quality of product has suffered even more than tonnage production up to now.

One of the principle illnesses of our industry is wide fluctuation in price during the year. The spread between average winter and average summertime price in 1952-53 fiscal year amounted to only $3.06, or 4.9 percent of the summertime price. But, the following year, this spread amounted to $24.20, or 56.7 percent, on the same basis of calculation. Spread between summertime low and winter peak is even greater. Such a situation militates against the fullest use of a fine primary farm crop of high nutritive value. The perishable nature of the crop, coupled with the fact that ninety percent of it is produced in fifty percent of the 12-month period, brings this situation about. Great strides have been made in the past three or four years in reducing the perishability of the product through storage under non-oxidizing atmospheres. There is now probably about 375,000 tons capacity in existence. This amount is just about equal to 6 months' wintertime disappearance, which has averaged 381,000 tons in the past five years. A further great advance on this front will be made when introduction of an effective anti-oxidant can be included in the production picture.

We estimate that two-thirds to three-quarters of all dehydrated alfalfa is now produced in pelleted form, though not all of it goes to market as pellets. We believe there is now more meal marketed in the form of ground pellets than in the form of originally produced meal. Much of the meal placed in commercial channels is treated with oil or grease, or with some modification, such as methyl-ester of cottonseed oil. This is done principally to lay the dust which is so objectionable to the user. A further benefit to the producer is in less loss of product from the stack of the cyclone collector.

We spoke earlier of a phase in our program which differs from many Trade Associations; our participation in research. Our program of research began in 1949 and, believe it or not, we lost some members because of it. There are even some dehydrators today who refuse to join us because of our spending for research. It is our firm belief, however, that without a program of research the steady increase in consumption of our products would have lost most of its acceleration and our Association would have been a much weaker and less effective tool.

Since the beginning of our research program in 1949, we have given grants-in-aid to 17 experiment stations and colleges, covering approximately 40 projects, a total amount of about $165,000, with about $48,000 additional for publishing alfalfa abstracts, for Research Council travel, and for administration.

The President's Bi-Partisan Commission on increased industrial use of agricultural products recognized the need for greatly expanded research in the particular field of agricultural products utilization. In a Washington release of April 18, it is reported that the manufacturing industry devotes an average of 3 percent of its gross annual income to research and development. The gross market value of dehydrated alfalfa for the fiscal year ending March 31st, 1957, amounts to a little over $58,000,000, arrived at by multiplying total production by average price per ton. Three percent of that dollar value is a million and a half. The American Dehydrators Association will spend almost exactly 1/100th of that amount on research projects during the coming year. Of course, that is not the end of the story. The amounts being spent by the colleges and experiment stations in carrying on the A.D.A. projects is very considerably in excess of the funds the Association gives to them. Also, the Association research activity has stimulated much additional alfalfa research entirely separate from sponsored projects. The amounts spent on alfalfa research through Agricultural Research Service of the Department of Agriculture, both in the field of agronomy and plant breeding, and in utilization, is no mean sum. We, in the dehydrating industry, like to think that we have played some small part in stimulating that research in certain areas of endeavor. Still, the amount our industry devotes to this very important phase is small when compared to amounts expended by other industries, particularly when it is considered that very little is spent by individual firms within the dehydration industry for their own private research.

Dehydrated alfalfa, grass, or clover is in no less vulnerable position than any other agricultural commodity. Actually, it is highly vulnerable to attack in certain segments of its trading area. Of special significance is the growth of the synthetic vitamin business, making it possible to supplant those with which dehydrated forage crops are blessed. We need further research on our own methods of drying in order to lessen the destruction of these factors, both during drying and storage. It might be well to call attention to a statement made by Dr. George Kohler, of this laboratory, in his address to the Association at the recent convention. He said,

"In alfalfa dehydration, rear-end temperature is related to quality only indirectly. Several years ago we found at Cerophyl laboratories that variability of loss of ascorbic acid, one of the most sensitive measures of product damage, is much more closely related to final moisture than to rear-end temperature. The dryer the final product was, the greater the destruction of ascorbic acid. Therefore, alfalfa can be over-dried and automation, based on continuous moisture measurement, would be much superior to that based on rear-end temperature. No plant operator wants to bog down his operation by clogging up his hammermill. Therefore, there is a strong tendency to over-dry meal. In addition to the harmful effect of over-drying on product quality--and this should be studied much more intensively--there are several other practical considerations which make it very desirable to produce and sell a meal containing at least 8 percent of moisture".

After naming several other advantages attached to different methods of control, Kohler cites a fourth advantage as follows, "A fourth advantage to be gained is that carotene stability in alfalfa is optimum at 7 to 9 percent moisture content. It may be that the lower stability at lower moisture level is due to destruction of natural antioxidant in alfalfa during over-drying".

Another factor which has caused industry to suffer loss of market is the growth inhibition, which has repeatedly been reported and cannot be wholly ignored. Under present practices in poultry feeding, it would seem that the inhibitor has little or no chance to make itself felt, but it should be remembered that it has played its part in loss of market. Growth inhibition in species other than poultry has been reported by some research workers. Can we, as an industry, afford to pass by any avenue of research endeavor which might lead to improvement of our product in the feeding field? Certainly we are not content with these low feeding levels of poultry rations today. Still further claims against the product are its high-fiber, low-energy values. The Association is spending some of its funds to have the energy re-evaluated at one of the experiment stations. A few firms within the industry are offering a portion of their production on a reduced fiber basis. Also, we are building a market with four-footed animals which can utilize the higher fiber, but wouldn't a study on methods of rendering fiber more utilizable be of considerable value to this industry? Either that sort of study or one to determine better methods of separating the fiber and then, perhaps, using it for entirely unrelated non-feed products. We cannot afford to take the attitude still held by a scattered few of our industry members who say in effect, "Here is our meal, it may not be exactly what you want. We are not equipped to oil and grease it, we don't have a pellet mill, nor can we provide you, therefore, with reground pellet meal, and we can't afford to put in gas storage in order to serve you the year 'round, but I've made and sold this stuff

for ten years, so I know it's what you need. I can't afford to put in all these new gadgets just to give you something a little different". That, my friends, is the beginning of the end for that producer. He is not thinking of what the trade wants, but only of what he makes. He has not yet realized that you either move with the demand or you start sliding downhill. Most of our customers know what they want and unless we provide it they'll look elsewhere.

This industry has made very rapid strides in the past several years in mechanical handling, improved physical conditioning of the product, and in reducing costs of operation. The ready availability of high-carotene values in the product throughout the full twelve months is a wonderful forward step achieved through storage under non-oxidizing atmospheres. Not enough emphasis has been given to the fact that this type of storage also conserves the other oxidizible factors, as well, such as vitamin E, vitamin K, and the pigmentation-producing xanthophylls. You will hear more of this later today.

This is only a delaying of the destructive oxidation of these fat-soluble factors, however. We need to go further, and not only deliver to the feed manufacturers, or feeders, a product of high vitamin value, but we also need to be able to assure him that those values will hold up during the time he holds it in storage, processes it, or actually until the bird or animal consumes it. In other words, we need a stable dehydrated product. This is only possible through stabilization with some chemical or other additive which will prevent, or greatly slow down, the degradation even under normal atmospheres. There is much need for study of the many carotenoid pigments to see which does the job and how their values may be enhanced. There is need to know the availability to the animal body of the tocopherols of alfalfa and how such availability may be improved. This applies also to carotene, particularly with respect to its vitamin A value to ruminants. There are some very remarkable claims being made for the biological value of dry vitamin A, as compared to carotene, from natural sources. Four-footed animals have used the natural source for centuries; surely, it can't be too bad, but in this era of intensive research, the mad scramble for the feed manufacturers' and the feeders' dollar forbids anyone the privilege of relaxation in research effort, just as a matter of survival in a highly competitive market.

The dehydration industry should greatly increase its research efforts along at least two lines; first, to find out more about the feeding value of its present products for all types of animals, and, second, to learn how present products can be improved in nutritional properties to better fit today's and tomorrow's demand for higher and higher production rates of red meat, poultry products and dairy products at lower and lower costs.

There may be some of you who feel that I have been unduly critical of the Research Program of our - my - Association. To you let me say that I believe no one has been more closely and continuously associated with this phase of our activities since its inception than have I. The research we have fostered, the advice and good counsel we have received from the many fine men of present and past Alfalfa Research Councils, the pleasant and satisfying relationships with project leaders over the nation are the greatest riches we enjoy today. Of all the activities of our Association the one which brings to us the greatest returns is our Research Program. From my personal standpoint, the greatest reward I have is from the successful prosecution of this particular activity. To me our very existence depends upon its continuation and, may I hope, its expansion in years to come.

A. D. A. AND OTHER ALFALFA RESEARCH

L. E. Card

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University of Illinois,
Urbana, Illinois

Mr. Chrisman has referred to research in telling you about the program of the American Dehydrators Association, and some of you are familiar with the several projects to which the association has been giving financial support. Since this conference is to be concerned very largely with reports of research, it is perhaps not out of order for me to review briefly the projects which are being concluded and others which are under way.

A project which has been carried out right here in California has to do with "Increasing the nutritive value and palatability of dehydrated alfalfa and oat forage by control of the fiber content through manipulating of harvesting intervals". This afternoon Dr. Meyer will give you some of the results of that study. The coming year's work is to be concerned with "Studies on the reasons for the high feed value of dehydrated and pelleted alfalfa as sources of energy and protein".

The Connecticut Station has been studying "The utilization of artificially dehydrated alfalfa as a feed for dairy cattle". Several papers have been published on this work and another is in press.

At the Cornell Station studies are being made of the energy value of alfalfa products for poultry. Results to date give metabolizable energy values for 14 samples of alfalfa meal that are considerably higher than were expected, with calculated productive energy values about twice what would be estimated from the earlier Texas data of Fraps. The Cornell work has also shown that neither the growth inhibitor as present in alfalfa nor added saponins at a low level of feeding interfered with the measurement of metabolizable energy.

The Illinois Station has been studying "Unidentified growth factors in alfalfa" as measured by chick growth experiments. During 1956 four different cuttings from two adjacent experimental plots were fed at 10 and 30 per cent levels in a cerelose-Drackett protein purified diet. Chicks on the basal diet without alfalfa averaged 449 grams at 23 days. But the addition of 10 per cent alfalfa meal consistently improved chick growth with no adverse effect on feed conversion. The addition of 30 per cent alfalfa depressed growth in each instance, but chicks fed the fourth cutting tolerated the high level very well, averaging 427 grams at 28 days.

The Kansas Station has concluded a study on "The marketing of dehydrated alfalfa".

An extensive study at Nebraska dealt with "Soil fertility in relation to the production of alfalfa for dehydration". Several reports have appeared and I shall not discuss them at this time.

A new project being started at Nebraska has to do with "The value of dehydrated alfalfa as a protein supplement (with and without stilbestrol) for fattening beef cattle".

The Washington Station has been studying the "Effect of various constituents on the utilization of roughages by pregnant beef heifers". During the past year gestating-lactating beef cows were fed 0%, 30%, and 60% of dehydrated alfalfa meal along with 85%, 55%, and 25% of ground wheat straw, and 15% of a barley-molasses concentrate. Birth weights of calves and their gain for the first two weeks were directly proportional to the level of alfalfa in the rations of their dams - 56 to 67 pounds for birth weight, and 69 to 98 pounds for average two-week weight.

These projects make up but a small part of the research being done on alfalfa, and I continue to be surprised - perhaps astounded would be a more appropriate word - at the variety of problems related to the production and utilization of alfalfa which are being studied by numerous research people. At the annual meeting of the American Dehydrators Association last January I listed fifteen widely varying topics which had been the subject of recent research. I believe that the list will bear repeating here.

1. Apparent resistance to the spotted alfalfa aphid selected from seedlings of susceptible alfalfa varieties.

2. A detailed study of the mechanics of feeding of the spotted alfalfa aphid.

3. Salt tolerance of alfalfa varieties.

4. Influence of additions of lime to soils on the availability of potassium and other cations for alfalfa.

5. Dehydrated alfalfa prevents scale in sea water evaporators.

6. Studies on the utilization of alfalfa by beef steers.

7. Inheritance of the white seed character in alfalfa.

8. Estrogenic activity of alfalfa and other feedstuffs.

9. Need of boron fertilization for alfalfa in Michigan and methods of determining this need.

10. Phosphatase test for determining heat treatment of alfalfa.

11. Utilization of high levels of alfalfa by growing-fattening swine.

12. Evaluation of pre-emergence and stubble treatments for control of dodder in alfalfa seed crops.

13. The estimation of the total digestible nutrients in alfalfa from its lignin and crude fiber content.

14. A comparison of the effect of complete and partial cross-pollination of alfalfa on pod sets, seeds per pod, and pod and seed weight.

15. Experiments in harvesting and processing alfalfa for dairy cattle feed - a 147 page technical bulletin of the U. S. Dept. of Agriculture.

The first six months of 1957 have been equally productive in terms of research reports on alfalfa. Without too much searching last week I found thirty different articles in fifteen different scientific journals, each on a different problem related to alfalfa. I am including them in this paper, with brief comments on a selected few, for the information of any persons who may be interested.

Ascarelli, I., and Bondi, A., 1957: The effect of different plant sources on the utilization of carotene by chickens. Jour. Agr. Sci., 49: 113-119.

Barker, M. G., Hanley, F., and Ridgman, W. J., 1957: Studies on lucerne and lucerne-grass leys. IV. The effect of systems of grazing management and the persistence of a lucerne-cocksfoot ley. Jour. Agr. Sci., 48: 361-365.

Bissell, T. L., and Ditman, L. P., 1956: Experiments on equipment and materials for spraying alfalfa for insects. Jour. Econ. Ent. 49: 636-638.

Bunnell, R. H., 1957: The vitamin E potency of alfalfa as measured by the tocopherol content of the liver of the chick. Poul. Sci., 36: 413-416.

Burdick, E. M., 1956: Extraction and utilization of carotenes and xanthophylls. Econ. Bot., 10: 267-279.

Cormack, M. W., and co-workers, 1957: Studies on methods and materials for testing alfalfa for resistance to bacterial wilt. Canad. Jour. Plant Sci., 37: 1-11.

Dobson, R. C. and Watts, J. G., 1957: Spotted alfalfa aphid occurrence on seedling alfalfa as influenced by systemic insecticides and varieties. Jour. Econ. Ent., 50: 132-135.

Ershoff, B. H., 1957: Beneficial effects of alfalfa and other succulent plants on the growth of immature guinea pigs fed a mineralized dried milk ration. Jour. Nutr., 62: 295-312.

Halstead, R. L., and co-workers, 1957: Phosphorus and potassium supply for alfalfa in soils sampled at different depths. Canad. Jour. Soil Sci., 37: 61-70.

Harper, H. J., 1957: Effect of rainfall and fertilization on the yield and chemical composition of alfalfa over a 10-year period in north central Oklahoma. Soil Sci. Soc. Amer. Proc., 21: 47-51.

Heinemann, W. W., Ensminger, M. E., Ham, W. E., and Oldfield, J. E., 1957: Effects of phosphate fertilization of alfalfa on growth, reproduction and body composition of domestic rabbits. Jour. An. Sci., 16: 467-475.

Hobbs, G. A., 1956: Ecology of the leaf-cutter bee Megachile perihirta in relation to production of alfalfa seed. Canad. Entom. 88: 625-631.

Howe, D. O., and Graham, E. R., 1957: Salt concentration a factor in the availability of phosphorus from rock phosphate as revealed by the growth and composition of alfalfa. Soil Sci. Soc. Amer. Proc., 21: 25-28.

Koehler, F. E., Moore, A. W., Allmaras, R. R., and Olson, R. A., 1957: Influence of past soil treatment on yield, composition, and fertilizer phosphorus utilization by alfalfa. Soil Sci. Soc. Amer. Proc., 21: 201-205.

Kratzer, F. H., and Davis, P. N., 1957: The effect of dehydrated alfalfa meal in the breeder ration on hatchability in turkeys and on the response of poults to alfalfa feeding. Poul. Sci., 36: 487-490.

Labanauskas, C. K., and Jackobs, J. A., 1957: Cork formation in tap roots and crowns of alfalfa. Agron. Jour., 49: 95-97.

Lesins, K., 1957: Cytogenetic study of a tetraploid plant at the diploid chromosome level. Canad. Jour. Bot., 35: 181-196.

McCaughey, W. F., Fleming, W. S., Vavich, M. G. and Kemmerer, A. R., 1957: Effect of heat treatment of alfalfa on availability of carotene to chicks. Poul. Sci., 36: 323-328.

Miller, M. A., 1957: Burrows of the Sacramento Valley pocket gopher in flood-irrigated alfalfa fields. Hilgardia, 26: 431-452.

Muka, A. A., 1957: Alfalfa weevil control with granulated insecticides in Virginia. Jour. Econ. Ent., 50: 216-218.

Nelson, W. W., and MacGregor, J. M., Effect of time and rate of fertilizer application on the yield, composition and longevity of alfalfa. Soil Sci. Soc. Amer. Proc., 21: 42-46.

Ogden, R. L., 1956: Effect of added fats and oils on carotene stability in dehydrated alfalfa meal during storage. Jour. Agr. Food Chem., 4: 428-431.

Peterson, M. L., and co-workers, 1956: Comparison of the chromogen and clipping methods for determining the consumption of dry matter and total digestible nutrients by beef steers on alfalfa pasture. Agron. Jour., 48: 560-563.

Porter, G. H., and Kesler, E. M., 1957: Value of alfalfa silage in the diet of the young dairy calf. Jour. Dairy Sci., 40: 163-172.

Rendig, V. V., and Weir, W. C., 1957: Evaluation by lamb feeding tests of alfalfa hay grown on low-sulfur soil. Jour. An. Sci., 16; 451-461.

Rodger, J. B. A., Williams, G. G., and Davis, R. L., 1957: A rapid method for determining winter hardiness in alfalfa. Agron. Jour., 49: 88-92.

Schonhorst, M. H., Davis, R. L., and Carter, A. S., 1957: Response of alfalfa varieties to temperature and daylengths. Agron. Jour., 49: 142-143.

Tesar, M. B., 1957: Establishment of alfalfa in wide-row corn. Agron. Jour., 49: 63-68.

Thomason, I. J., and Sher, S. A., 1957: Influence of the stubby-root nematode on growth of alfalfa. Phytopathology, 47: 159-161.

Touchburn, S. P., Biely, J., and March, B., 1957: The effect of dehydrated green feed on fertility and hatchability of eggs from three generations of New Hampshire chickens. Poul. Sci., 36: 591-595.

In the work of Rodger and co-workers, seed lots of different alfalfa varieties were germinated in solutions of sodium chloride and sucrose having known osmotic pressures. As the pressures increased, the speed and amount of germination decreased in all varieties, but the decrease was much more

marked in seed of the hardy varieties than in seed of the nonhardy ones. 75° F. proved to be a satisfactory temperature for the tests.

Schronhorst and co-workers grew ten varieties of alfalfa and a mixture of African and Ranger at temperatures of 60°, 70° and 80° F. under 8-, 12- and 16-hour daylengths for each temperature in constant temperature chambers. Growth responses showed the greatest height differences at 60° and 12 hours. At this temperature the varieties and mixture could be divided into three groups according to magnitude of the variances.

Group 1 (Shortest)

Vernal, Ranger, Narragansett, Grimm

Group 2 (Intermediate)

Buffalo, Atlantic, DuPuits

Group 3 (Tallest)

African, Mixture of African and Ranger

Miller's report from California on the pocket gopher pointed out that soil moisture is the major factor regulating gopher burrowing. In alfalfa fields the optimum range was 15-17 per cent moisture, with markedly less burrowing done under wetter or drier conditions. Distribution of burrows was such that control by gassing is theoretically unsound. Poison baits are better. Subsoiling to a depth of two feet would give practically complete destruction of nests and burrows and thus discourage reinvasion.

Touchburn, Biely and March fed dehydrated alfalfa meal and dehydrated cereal grass to New Hampshire chickens from the time of hatching through a period of egg production. A second and a third generation of chickens from the original experimental groups were given the same treatments in which alfalfa and cereal grass fed at levels of 2.5 and 5 per cent of the ration were compared with a control ration containing neither of these products. All three generations were started in battery brooders, and at eight weeks of age transferred to floor pens and raised on deep litter.

Fertility of eggs for 10 hatches in 1954 and for 6 hatches in the two following years averaged 97 per cent for all groups, with only slight variation (94.3% to 98.1%). Hatchability of fertile eggs averaged 87 per cent. There was a slight, but significant, improvement in hatchability from the dehydrated green feeds.

Kratzer and Davis fed turkey hens during two different years on rations containing 5, 15 and 30 per cent of dehydrated alfalfa meal, and for a third year on rations containing 5 or 30 per cent. All experiments were started in late January and eggs were set from mid-February to early June. They found no detrimental effect on egg production from the high level of alfalfa and no reduction in hatchability. The alfalfa meal used in 1956 was known to give marked retardation of growth, but poults hatched from dams fed 30 per cent of this meal grew as well as those from hens fed lesser amounts or none at all. On the other hand, either 10 or 20 per cent of this alfalfa in a starting ration for poults caused significant growth depression which was independent of the type of ration fed to the dams.

All of this research is encouraging in that it helps point the way to more effective production and utilization of alfalfa.

IMPROVING ALFALFA THROUGH RESEARCH

H. O. Graumann

Crops Research Division
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During the first three quarters of a century of alfalfa culture in the United States production hazards were at a minimum. But, as experienced with other crops, with the passing of time from original introduction, and as acreage and area where grown increased for alfalfa certain diseases and insects loomed as production hazards. Today there are several score of these against which some control, either inherent plant resistance or chemicals applied to the crop, is needed for specific production areas if alfalfa is to be grown successfully. Such controls as are currently available came about as result of comprehensive research. In my discussion today, reference will be made to only a few of the problems and the successes attained from research designed to solve them. Progress reported includes combined efforts of plant breeders, plant geneticists, plant pathologists, plant physiologists, and entomologists.

The first widespread serious threat to alfalfa was observed in the mid 1920's. Stands were noted to be dying much sooner than previously experienced. Careful study showed that a disease was involved which later was described as bacterial wilt. Initiation of work to breed for resistance to this disease marked the beginning of comprehensive alfalfa breeding and improvement research in this country. Breeding principles which formed the basis of procedures developed as this program progressed are basic in any breeding program. One basic requisite which soon became apparent and still holds is that the speed and relative success of accomplishing a breeding objective is dependent largely upon the development of quick, reliable and large-scale methods of appraising plant materials for the character in question. In the case of bacterial wilt, thousands of plants were inoculated each year with pure cultures of the causal bacteria for the purpose of isolating plants with resistance.

Because of initially low levels of resistance to bacterial wilt, a recurring system of intercrossing selected plants and screening the resulting progenies for resistance in controlled bacterial wilt tests was used to build up the frequency of genes conditioning this character. Several cycles of this recurrent selection procedure were required to attain suitable resistance to this disease, hence progress was slow, but nevertheless very effective. The first improved bacterial wilt-resistant variety was released in 1942. Today there are available to American farmers five varieties with resistance to this disease, namely, Ranger, Buffalo, Caliverde, Vernal and Lahontan. These have an estimated annual value of more than $100,000,000 to American agriculture. Research work in developing additional bacterial wilt-resistant varieties is continuing, with many thousands of plants being appraised each year for reaction to this disease.

Breeding for resistance to the stem nematode started in 1940. The great challenge in this work was to incorporate into adapted and otherwise superior stocks resistance to both stem nematode and bacterial wilt. This was accomplished in the development of the variety Lahontan which was released in 1954. This variety is fulfilling critical needs in many of the stem-nematode-infested, irrigated valleys in Western United States.

Various stem and foliar diseases of alfalfa, especially black stem and the leaf spots, cause serious yellowing and dropping of leaves. These diseases are most destructive in the more humid sections of the country. Damage to and loss of leaves from these bring about a serious reduction in both yield and quality of forage. Breeding for resistance to these diseases has been difficult. To date there are no varieties with sufficient levels of resistance to give complete protection against damage from the host of causal organisms. The search is continuing for breeding stocks with high levels of resistance. Because of the many organisms involved a sound and effective breeding program must take into consideration the problem of multiple resistance to leaf and stem diseases.

Potato leafhoppers usually cause stunting of plant growth and extreme yellowing of leaves, particularly in the second and third cuttings of alfalfa each year. While satisfactory spray programs for their control have been developed many farmers because of extra labor and cost are reluctant to use them. Breeding for resistance to this insect has advanced to a stage that several promising experimental combinations have been synthesized and are currently under test. It will take several years to determine whether the level of potato leafhopper resistance is adequate and other features of the alfalfa combinations good enough to warrant release.

The most recent and probably most serious production hazard to ever face the alfalfa industry in the United States is the spotted alfalfa aphid, an insect that in 1956, the third year after its initial discovery in this country, was found in 30 of the 48 States and caused in excess of 40 million dollars' damage. Breeding for resistance to this pest has advanced at a phenomenal rate. The variety Lahontan, released in 1954, is highly resistant to this pest. Resistant parent lines from this variety, and additional resistant stocks isolated from other sources have been used in the development of new spotted alfalfa aphid-resistant combinations. Several of these are currently under test. The most promising of this group is Nevada Syn M, a winter tender, vigorous, quick recovering type involving spotted alfalfa aphid resistant plants selected from the susceptible variety African.

The changes in techniques used in appraising plants for reaction to the spotted alfalfa aphid illustrate how advances in procedures facilitate in the development of more effective breeding programs. In the early stages of breeding for resistance to this pest plant reaction to the insect was determined by enclosing individual shoots in small cages used for confining known numbers of aphids to the plant and excluding predators. About a year ago a further advance was made by the use of screened houses ranging in size from 100 to 400 square feet. A single facility of this type provides enough space for planting short rows of a number of alfalfas

and permits the introduction of aphids in sufficient numbers to determine quickly which stocks, if any, have economically satisfactory levels of resistance. Concurrent with this it was discovered that appraisal of plants in the seedling stage speeded up the program. The most recent technique for mass plant appraisal involves the planting of plots in the field and using selective insecticides which will control the predators and not have any harmful effect on the spotted alfalfa aphid, thus permitting populations of the aphid to build up to economically damaging proportions in short periods of time.

Over the years competition from weeds during the year of alfalfa establishment has been a serious problem, particularly in spring seedings. Research currently under way with selective herbicides for control of weeds in seedling stands of alfalfa shows great promise. Preliminary and inconclusive results indicate that certain materials used as pre-emergence sprays will control broad-leafed weeds and weedy grasses for several months without impairing emergence or growth of alfalfa. More research is needed to confirm preliminary findings.

Another benefit derived from the improved disease-resistant alfalfas is their greater flexibility of management. Recent studies in Wisconsin[1/] covering a period of four years show that Grimm, one of the old standby varieties, when cut 3 times annually produced 8% less forage and deteriorated in stand much sooner than when cut twice. The varieties Ranger and Vernal in this same test produced 22% and 3% more forage, respectively, from 3 cuts than from 2 without any apparent reduction in stand. Moreover, the protein yield under the 3 cut regime was 52% higher for Ranger and 25% greater for Vernal. This greater flexibility in management is extremely important to the farmer who oftentimes, because of other pressing farming operations, finds it impractical to follow the rigid harvest schedules required for the old highly-disease-susceptible varieties. Also, it permits harvesting at slightly earlier stages of plant development before protein and carotene contents show a sharp decline, either because of disease and insect damage to the leaves, or advancing plant maturity.

The work in breeding for resistance to various diseases and insects is continuing insofar as funds and facilities permit. Past accomplishments serve as evidence of advances that may be expected in the future. When considering the extreme plant-to-plant variability that exists in alfalfa there is reason to believe that progress can be made for any given character for which quick and reliable methods for appraisal or assaying can be developed.

1/ Smith, Dale. Cutting Alfalfa Early for Better Quality Hay and Silage. Wisconsin Agr. Exp. Sta. Bul. 521:1-4, 1956.

BIOLOGICAL AND CHEMICAL CONTROL OF SPOTTED ALFALFA APHID

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We are confronted with many unwanted insects which have arrived in this country free of their complement of natural enemies. One of the lines of investigation followed by workers in biological control is to determine as near as possible the original home of undesirable alien pests, to then explore these areas in an effort to find natural enemies. If and when desirable enemies such as parasites, predators and pathogens are found the next step is to successfully introduce and establish them in this country.

When the spotted alfalfa aphid invaded and began to spread over the United States it was found that many of the existing predators would feed upon them and fortunately in some areas predators and pathogens became very important factors of control.

There were, however, no parasites of importance found in the existing complement of natural control factors. In order to rectify this situation the University of California, Department of Biological Control, sent Dr. Robert van den Bosch on an exploratory journey to the Mediterranean Area, and to the Near East. As a result of his efforts three species of parasites, Praon palitans, Trioxys utilis and Aphelinus semiflavus, were discovered.

The newly discovered parasites were shipped to Riverside, California, and after their arrival they were released from quarantine and a program of insectry breeding was initiated. Within a short time many thousands of these parasites were obtained for field release in southern California. By midsummer of 1956 it was apparent that the introduced parasites had become well established as they could be readily recovered many miles from the original point of release.

Insectary mass production in northern California got under way at Albany during the spring of 1956. The material produced there was used for colonization in California, and also to furnish parasites for experimental purposes to other states. To augment the California production, parasites were also reared in the Entomology Research Division's station at Moorestown, New Jersey. Material from both sources was furnished to Arizona, Arkansas, Nevada, New Mexico, Missouri, Oklahoma, Texas and Utah. Due to the great increase in the number of infested states the distribution program has been reorganized in 1957 so that all of the states east of the Mississippi River are handled out of Moorestown, New Jersey, and those to the west from Albany, California.

Generally speaking the accomplishment phases associated with a parasite project can be divided into: Introduction, Colonization, Establishment and Control. Currently our major interests in the northern San Joaquin and the Sacramento valleys are with colonization and recovery, whereas in southern California the interest is shifting to a study of the effectiveness of the parasites which are now well established.

In states other than California the major emphasis is also introduction and colonization. Recoveries have, however, been made in Oklahoma, New Mexico and Arizona, and we hope that soon they will become sufficiently abundant in Arizona so that no further colonization is needed.

The parasite distribution in California during 1957 has been greatly accelerated by the rapid increase of parasites in the field and by the use of a vacuum collector devised by R. L. van den Bosch and associates. This collector is mounted on a light truck which is driven through fields having heavy parasite populations. A few trips across a field will pick up more parasites than could be produced in the insectary in a year. The parasites collected by the University of California are distributed locally by ranchers, while distribution between the various counties is handled by the University of California in cooperation with the Agriculture Commissioners and the Agriculture Extension Service.

During the past year information has been obtained on some very important questions such as: will the parasites survive through the four seasons, will they have a rapid increase, and will they be able to disperse over large areas.

During the winter months which has been thought would be the most critical season, Trioxys and Aphelinus continues to reproduce at a reduced rate, and there does not appear to be very pronounced cessation of activity, while Praon has a definite diapause or resting stage.

Aphelinus, in the areas observed, so far has not shown as much ability to increase as has Praon and Trioxys. The millions of parasites collected this year have originated from one and two year old colonies and they have been predominately Praon and Trioxys.

Dr. van den Bosch reports that by last fall (1956) in the Antelope Valley Praon had spread over a 400-square mile area while Trioxys had spread over 5 to 10 square miles. He believes that the dispersion of Praon is greatly assisted by its being carried by parasitized winged aphids. All three species have been able to disperse quite well as adults. In fact it is believed that their rate of dispersion has made it difficult to obtain large build-ups in the immediate area of release. This rapid rate of dispersion makes recovery records difficult to obtain until the populations

of parasites have reached a fairly high level. It is just as likely that a recovery can be made a mile away as in the immediate vicinity of a release. Therefore, it is readily seen that the area to be searched is quite large in relation to the number of newly established parasites.

In discussing the chemical control aspects comments will be confined to the possibilities of integrating chemical and natural control. The last word on this subject is far from being spoken, because every day something new is learned. Universally applicable recommendations cannot be made because every unit of growing alfalfa presents problems unique to that unit.

There are indications that there is some resistance to parathion on the part of the spotted alfalfa aphid. This condition is reported in the University of California, Agriculture Extension Service circular, "The Spotted Alfalfa Aphid and its Control in California". This tendency to resistance brings up a subject upon which we can generalize and that is to not treat unless it is absolutely necessary because excessive treatment could accelerate the development of resistance not only to parathion but possibly to other organic phosphate insecticides. One of the best pieces of advice on the use of insecticides is contained in a paragraph taken from a previously mentioned Extension circular: "Preventative treatments directed against low non-economic aphid populations only aggravate the control problem by eliminating populations of natural enemies which tend to hold the aphid in check. On the other hand, it is not advocated that an alfalfa grower allow his fields to suffer economic damage from the aphid. When necessary, the wise and considerate use of insecticides, proper timing of application and correct dosage are just as important a part of good alfalfa hay production as is a proper irrigation schedule, or the cutting, baling and marketing, or feeding of the crop".

When is it necessary to treat? This is a topic which can not be generalized, as the decision to treat when you have certain numbers of aphids present is dependent on whether you have seedling or older alfalfa, or are growing a seed crop, and the area in which you grow alfalfa, and the time of year. In California the alfalfa growing areas have been classified, on the basis of the aphids habits, into three main groups, and the number of aphids per stem at which treatment should be considered has been determined for these groups.

Regardless of where alfalfa is grown the fields should be kept under constant observation to ascertain the presence or absence of natural enemies and to determine any changes in aphid populations by making actual stem counts.

With the continuance and advancement of the intensive studies on insecticides, natural enemies, ecology and plant resistance, I think we can look to a brighter future as far as the spotted alfalfa is concerned.

/aphid

MECHANICAL UPGRADING OF DEHYDRATED ALFALFA

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Because of the construction of the growing alfalfa plant, as differentiated from the grasses, it is possible to make a fairly good separation of the leafy part from the stems by mechanical methods.

In drying the plant, the leaves, which are of much thinner section, dry first and the stem, of heavy cross section, is slower to give up its moisture.

The nature of the composition of the plant is such that the leaves, when dried, are much more friable than the stem; more easily shattered or broken up.

It is well known, of course, that the leaf portion is richest in protein, carotene and most, if not all, of the nutritionally important constituents. The stem is low in these factors, but carries the major part of the fiber.

These differences in plant structure, coupled with the differences in friability and nutrient content, makes it peculiarly susceptible to mechanical fractionation along the lines of nutritive values.

This is one of the reasons why hay cut from the same field at the same time will not run as high in protein as the dehydrated product. Shattering of the leaves during natural drying, turning, raking and baling will result in lower protein and higher fiber.

In the earlier years of dehydration many plants were equipped with sifting devices of one kind or another. They were not used continuously. If a producer had a carload of 17% meal sold and committed for a specific shipping date and then found himself unable to get anything above 16% in the field, he could run his production through the sifter, take off a portion which ran 17% to 18% and would meet his commitment. Of course, he was left with another fraction which might run 14% to 15% protein. This could either be sold at a lower price or could be stored for purposes of blending it late in the season with some high quality fall production to make a 17% product.

The same facts and principles might now be used, and in some instances have been used, to try to provide a more quality-demanding feed trade with an upgraded product, principally for inclusion in broiler feeds. Broiler feeds have become such highly scientific concentrated sources of chicken meat builders that anything but a bare minimum of any substance which will not contribute to that phenominal growth rate is frowned upon.

Presented at Fourth Technical Alfalfa Conference, jointly sponsored by American Dehydrators Association and Western Regional Research Laboratory, Agricultural Research Service, Albany, California, July 26, 1957.

There are still many outlets for the 17% protein - 100,000 I.U. dehydrated product which has become the most nearly standard product we have. There are also outlets in ruminant feeding for somewhat lower grades.

Most of our alfalfa today is pelleted as produced. Much of the meal sold today is made by breaking down those pellets by putting them through a hammer mill, attrition mill or impact-type grinder. The fact that the alfalfa has been ground and pelleted and the pellets, in turn, ground, still does not rule out mechanical upgrading in processing the reground material.

The experience of one firm in doing this has been given to me and it is essentially as follows:

A blend of pellets to give an average of 18% protein and 120,000 I.U. vitamin A is used here as an illustration.

These pellets are broken down in an attrition mill or entoleter and sent to a screen having two mesh sizes, - 20 mesh on the first screen and 80 mesh on the second, thus there will be three streams from the screening.

The plus 20 mesh, material which will not pass through, is recirculated to the breakdown mill so that eventually it all passes through.

The material which passes through the 20 mesh, but will not pass through the 80 mesh, is in the form of granules and will constitute about two-thirds of the total product. Adjustments in the grinding can be made to assure this two-third yield at that point.

The material passing through the 80-mesh screen is, of course, the more friable part of the total. It will constitute one-third of the total product.

What happens to the quality when starting with an 18% pellet?

The two-thirds which is minus 20 plus 80, or, in other words, passed through one screen but not the other, will run about 17% protein and about 110,000 I.U. vitamin A and would, therefore, be saleable as the standard product.

The one-third which passes through the 80-mesh screen will analyze about 20% plus protein and in the neighborhood of 125,000 to 140,000 I.U. vitamin A. This fraction will contain about 20% fiber.

We are of the opinion that capital investment to perform such an operation would run in the neighborhood of $17,500, in order to accommodate approximately 8 tons per hour.

Our same informants have experimented on a commercial scale with another system of upgrading. This involves using the type of long cylindrical screens, such as Bran finishers, where the cylinder remains stationary, but the material is brushed against it in passing through. By this method of treating the freshly dehydrated, but not ground, material from the dehydrator, it is possible to get a separation of stemmy material prior to any fine grinding and pelleting.

Again starting with an 18% protein alfalfa, the separation is about one-fifth oversize and four-fifths undersized. In other words, about 80% of the product goes through the screen and 20% comes out as stems.

When starting material is 18% protein, the one-fifth we call stems will analyze about 9% protein and the four-fifths about 22% protein.

By subjecting this four-fifths portion, at 22% protein, after pelleting to the same treatment first mentioned, i.e., milling and sifting, it is possible to come out with two-thirds of the product analyzing 20% protein and one-third analyzing about 25% protein.

It must be understood, of course, that these operations all add to cost of product; that, as superior products are made, they must be sold at a premium to offset the reduced price of the inferior byproduct. Also, the cost of the operation itself must be regained through adequate price obtained for all products.

INFLUENCE OF STAGE OF MATURITY ON THE UTILIZATION OF ALFALFA

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Two years ago at a meeting of this group the University of California reported on their experiments regarding the influence of the fiber content on the utilization of alfalfa. It was reported that alfalfa, holocellulose, hemicellulose, and lignin were not utilized by the white rat. Ruminants, however, were able to utilize the two cellulose components but lignin interferred with the utilization of cellulose by the rumen microorganisms. Other experiments were reported to show that digestibility did not always indicate the proper value for alfalfa. Animals fed high quality alfalfa made relatively greater use of the total digestible nutrients than when the total digestible nutrients were supplied by poorer quality alfalfa.

The purpose of this paper today is to explore further the importance of lignin on the total digestible nutrient content of alfalfa and on the influence of stage of maturity on the composition, and total digestible nutrient content of alfalfa, and its utilization by steers and sheep.

An estimation of the nutritive value of roughages from a simple chemical analysis would materially add to the precision of formulating rations for ruminants. Lignin has been used successfully by some workers to estimate energy or organic matter digestibility. However, there has been some lack of agreement that primarily seems to be due to variation in the plant species used. Crude fiber has often been used but correlation between crude fiber content and digestibility has not been as satisfactory.

In our study, latin-square designed digestion trials were conducted on 31 samples of alfalfa hay fed in 152 digestion trials. Total digestible nutrient content was calculated and correlated with the lignin, crude fiber and nitrogen content of the alfalfa. It was found that the correlation coefficient was -0.88 for lignin, -0.86 for crude fiber and 0.77 for nitrogen. An inspection of the standard errors of estimate indicate that lignin was the most satisfactory followed by crude fiber and nitrogen. Crude fiber was almost as reliable as lignin. This is probably due to the fact that a large portion of lignin is recovered in the crude fiber of alfalfa. Consequently it seems that crude fiber might be the constituent of choice when predicting TDN content of alfalfa.

It should be emphasized that these regression equations apply only to alfalfa or plant species which would have a similar site and progress of lignification.

In other studies daily samples of alfalfa were collected from fields of alfalfa from early pre-bud to full bloom in two different years. Lignin level in alfalfa changed slightly between early pre-bud and early bud. After this time a great increase in lignin was noted to the 10% bloom stage. Here the lignin content plateaued. From the 10% to the full bloom stage the lignin content was relatively constant. In the second year this period was not as extended as that found in the previous year. Protein inversely followed similar patterns. The reason for the plateauing of lignin and protein content after bloom is probably due to a recurrent growth and because the elongation and increase in stem size does not change greatly after blooming starts.

It should be pointed out that the bloom stage was determined by hand count of the stems and any stem with a blossom was considered to be in bloom. Consequently, 10% bloom means that 10% of the stems had at least one blossom or more.

In 1955 it was found that the feeding trial data with dehydrated alfalfa were in general agreement with the conclusion that might be reached from the changes of lignin content of the alfalfa. With the exception of the group fed 46% bloom alfalfa, daily gains seemed to be highest at the pre-bud and bud stages and somewhat lower in the 10% and full bloom stages. There seemed to be a variation due to food intake. When the daily gains were adjusted to an equal food intake by means of regression it was found that the daily gains were somewhat equal from pre-bud to the 62% bud stage. Then they declined and were equivalent after the 10% bloom stage.

The total digestible nutrient content showed a similar pattern in that the digestibility was highest in the bud stages and declined to the 10% bloom stage and then did not vary.

Maximum yield of dry matter for the season was obtained on the 10% and 46% bloom alfalfa. Production of digestible protein per acre for the season was much greater from the 1%, 62% bud and 10% bloom alfalfa. There was a distinct drop when the 46% bloom stage was reached. TDN yield was about the same for the 10% bloom and the 46% bloom alfalfa. Lamb production per acre, however, seemed to be about equal at 1% bud, 62% bud, 10% bloom and 46% bloom, however, since the greatest number of fat lambs were produced from the bud alfalfa feeding, it seems that the greatest energy production was from the bud stages.

Results of 1956 show a similar picture. The dehydrated alfalfa was fed as chopped and as pelleted alfalfa. The greatest weight gains were from the lambs fed 16% bud and 2% bloom alfalfa. Thirty-four percent and 100% bloom alfalfa produced lower daily gains. This was true whether the alfalfa was fed in the pelleted or chopped form. It seems the point around which dehydrated alfalfa changes is 10% bloom. It is at this point that the alfalfa decreases in value enough to show significant differences in weight gains and efficiency of utilization of alfalfa.

A steer trial was conducted in 1955 with sun-cured bud stage, 10 and 50% bloom alfalfa. Barley was fed to part of the steers fed each stage of maturity. It was found that significantly greater daily gains were made by the steers fed bud stage alfalfa versus those fed 10 and 50% bloom alfalfa. There was no difference between 10 and 50% bloom stage alfalfa in their ability to produce daily gains. Efficiency of feed utilization was greater for the bud stage alfalfa with no difference between the 10 and 50% bloom alfalfa. These data agree very well with previous results with dehydrated alfalfa fed to sheep. Further studies, however, with sheep fed sun-cured alfalfa the following year, did not show the marked differences between the various stages of maturity as found with the steers in this study or the study with sheep fed dehydrated alfalfa. Further work is needed to clarify this situation.

In general it seems that dehydrated alfalfa with highest nutritive value as an energy source is that in the bud stage. About 62% bud alfalfa will produce maximum yields of alfalfa in terms of energy equivalent as measured by weight gains. However, greater yields of dry matter were obtained from 50% bloom alfalfa. It seems that the turning point in the advancing stage of maturity of dehydrated alfalfa is at 10% bloom. After this time the value of dehydrated alfalfa as an energy source has decreased to/relatively constant level. In all cases it seems that lignin is the best chemical constituent in alfalfa that can be used as a reliable means of evaluating alfalfa as an energy source. Crude fiber is also a valuable tool to use in evaluating alfalfa.

COMPREHENSIVE ANALYSIS OF DIFFERENT QUALITY DEHYDRATED FORAGES

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In common with most other words, "composition" means different things to different people. To the plant physiologist, a plant is composed of morphologically distinct parts such as roots and stems, petioles, veins, vascular bundles, stomata, etc. To the cytologist, the plant is composed of various types of cells, one type of which is shown on slide 1. It contains a wall, a series of membranes, a mass of cytoplasm and a group of organized bodies, including the chloroplasts, the nucleus, starch granules, vacuoles, and mitochondria. The chemist is interested in still smaller units--the chemical compounds which make up the plant as a whole and its various parts. The chemist must correlate his results with those of the plant physiologist and the cytologist. The tremendous variability he encounters in plant material arises for the most part from (1) variations in growth rate due to soil and weather and (2) differences in stage of maturity.

Our knowledge of the location in the cell of the various chemical constituents is as yet very fragmentary. It might be of interest, however, to mention a few of the points which are generally agreed upon by plant chemists. The wall and cementing materials between cells are composed of the materials to which I shall later refer as "structural components"--the cellulose, lignin, pectin, and hemicelluloses. The bulk of the cell protein is in the gel-like cytoplasm and its inclusions, the chloroplasts. The latter also contain the great bulk of the fatty materials of the leaf, including essentially all of the carotene, xanthophylls, chlorophyll, vitamin K and vitamin E. It is in the chloroplasts that the marvelous photosynthesis reaction is carried out.

Chemical interest in forage crops is focused primarily on those components of the whole aerial portions of the plants which have beneficial or deleterious effects on their value as feedstuffs. The second slide shows why the forages are the most important group of crops grown as the basis of our livestock industry. As pasture and range, the 100 million tons of forages produced annually supply more than half of the total feedstuffs consumed. Forage crops harvested at an immature stage of growth are so rich in carotene, vitamin E, vitamin K, and xanthophylls that, properly preserved, they form a primary concentrate for mixed feed supplementation.

Of the forage crops, alfalfa is the leader since it is outstanding in nutritional quality, it can be produced economically on a large scale, and it fits well in crop rotations. Thus, alfalfa constitutes over half of the total forages produced in this country or over a fourth of the total feed consumed.

On the next slide are shown some of the shortcomings of alfalfa as a feedstuff. As a ruminant feed, its chief drawback lies in the fact that it sometimes causes bloat in cattle and sheep. As a poultry supplement the amount of alfalfa which is used is limited (1) by the fact that it is low in digestible energy and (2) by the fact that some lots of alfalfa cause growth inhibition when fed at high levels.

In my discussion of the composition of alfalfa I should first like to review briefly what we know about alfalfa constituents. Secondly, I shall present limited data showing the variability of selected components. Finally, I shall show a few of the correlations which exist between selected components.

The next slide shows what is known as a "proximate" or feed tag type of analysis of alfalfa meal. Not a single item on this slide refers to a specific chemical entity.

The crude protein as I shall show later is correlated in fresh meal with true protein, carotene, and xanthophyll. The crude fiber is inversely correlated with total digestible nutrients, although the degree of correlation is too low to be of much use in predicting feeding value. Ash contents greater than the normal give some idea of the amount of top soil scooped up by the harvester. N.F.E. is very roughly related to the energy value. Thus, there is some value to this type of analysis even though it has very severe limitations.

Both scientist and feeder need much more detailed knowledge. In the next series of slides I have compiled a summary of some of the constituents of the crude composites shown by the proximate analysis. From the next slide it is seen that the "crude protein" contains about 30% of non-protein material about 60% of which, in turn, is composed of free amino acids. Of the remaining 12% only a few compounds have been identified such as stachydrine, an alkaloid, and choline and betaine, both of which have some nutritional value. A small amount of the residual nitrogen is in the form of water-soluble B vitamins which will be discussed later.

In the next slide are shown the essential amino acid components of alfalfa meal. The proteins of the various leaf materials are amazingly consistent in amino acid content regardless of their source. Thus the analysis shown applies to grasses, clovers, spinach, etc., just about as well as it does to alfalfa. Another interesting observation is that they bear a striking resemblance to the protein of heart muscle. These similarities are undoubtedly due to the fact that leaf proteins are protoplasmic proteins as are heart and muscle proteins. The proteins ordinarily referred to as plant proteins and considered to be unbalanced from a nutritional point of view are the seed proteins which are storage materials in contrast to protoplasmic materials later to be broken down before use by the germinating seed. Thus, from the standpoint of amino acid balance, the leaf proteins are of a very high quality.

The non-essential amino acids of alfalfa are included in the next slide for completeness. No comments are necessary except that the ruminant can use them effectively.

In the next slide are shown some of the constituents of the crude fat fraction of alfalfa meal. True fat, or triglycerides, constitute less than half of the crude fat fraction. Of particular value here are the fat-soluble vitamins and the xanthophylls. I say xanthophylls since we know that at least 8 or 10 different compounds are included. A major one, lutein, and possibly others are important in producing pigmentation in the skin and shanks of broilers and in the yolks of eggs. The wax fraction of alfalfa is almost completely indigestible. It seems possible that it may actually interfere with the absorption by animals of the fat-soluble vitamins and xanthophyll. The chlorophyll is comprised of about 75% chlorophyll a and 25% of the blue-green chlorophyll b. While many claims have been made for pharmaceutical and cosmetic uses of chlorophyll, no consistent large-scale market has as yet been developed.

Vitamins E and K supplied by dehydrated alfalfa are of importance in the poultry and livestock industry. Some question has been raised as to the availability of the vitamin E in alfalfa. The Western Regional Research Laboratory is collaborating with the American Dehydrators Association, the Wisconsin Alumni Research Foundation, Cornell University, and the University of Illinois to find out whether these claims are valid. Results to the present indicate that essentially all of the vitamin E of alfalfa meal is available. An extensive survey of the amounts of vitamin E and vitamin K in alfalfa meals from various sources has been carried out at Colorado State University under contract from the Western Regional Research Laboratory. Dr. Thornton will describe some of the results in the next presentation.

In the next slide I have shown some of the components of the ash fraction of alfalfa meal. The ash of alfalfa is an important contributor to its value as a ruminant feed. While this analysis shows many constituents, we know that there are important elements in alfalfa ash not yet isolated. Synthetic mixtures with these minerals do not have the same effect in stimulating cellulose digestion as do the whole alfalfa or alfalfa ash. Perhaps the missing factor is selenium or bromine since both have been reported recently to be of importance in poultry nutrition. Molybdenum may also be involved, although too high an intake of this element in forages in England has been shown to cause a disease in cattle called "Teartness".

The following slide shows some components of the "crude fiber" and "N.F.E." fractions. These are combined since some of the original structural elements found in the plant appear in both places in the proximate analyses. Thus the celluloses appear in both fiber and N.F.E. fractions as do the lignin and hemicelluloses. This whole field of structural compounds is in a confused state at present so that much more work is needed before the digestibility of forages can be evaluated from chemical analyses. Cellulose can be digested by ruminants. Lignin, however, interferes with cellulose digestion. Ash constituents as mentioned above stimulate cellulose digestion. These complications together with lack of chemical specificity make "crude fiber" and "N.F.E." analyses almost meaningless as a measure of energy values of forages.

The organic acids are usable as a source of energy by animals. Our interest in them has been restimulated by the work at the University of Wisconsin which indicates that they may be important contributors in gas formation to cause bloat in cattle and sheep. We are currently reinvestigating the organic acids of forages.

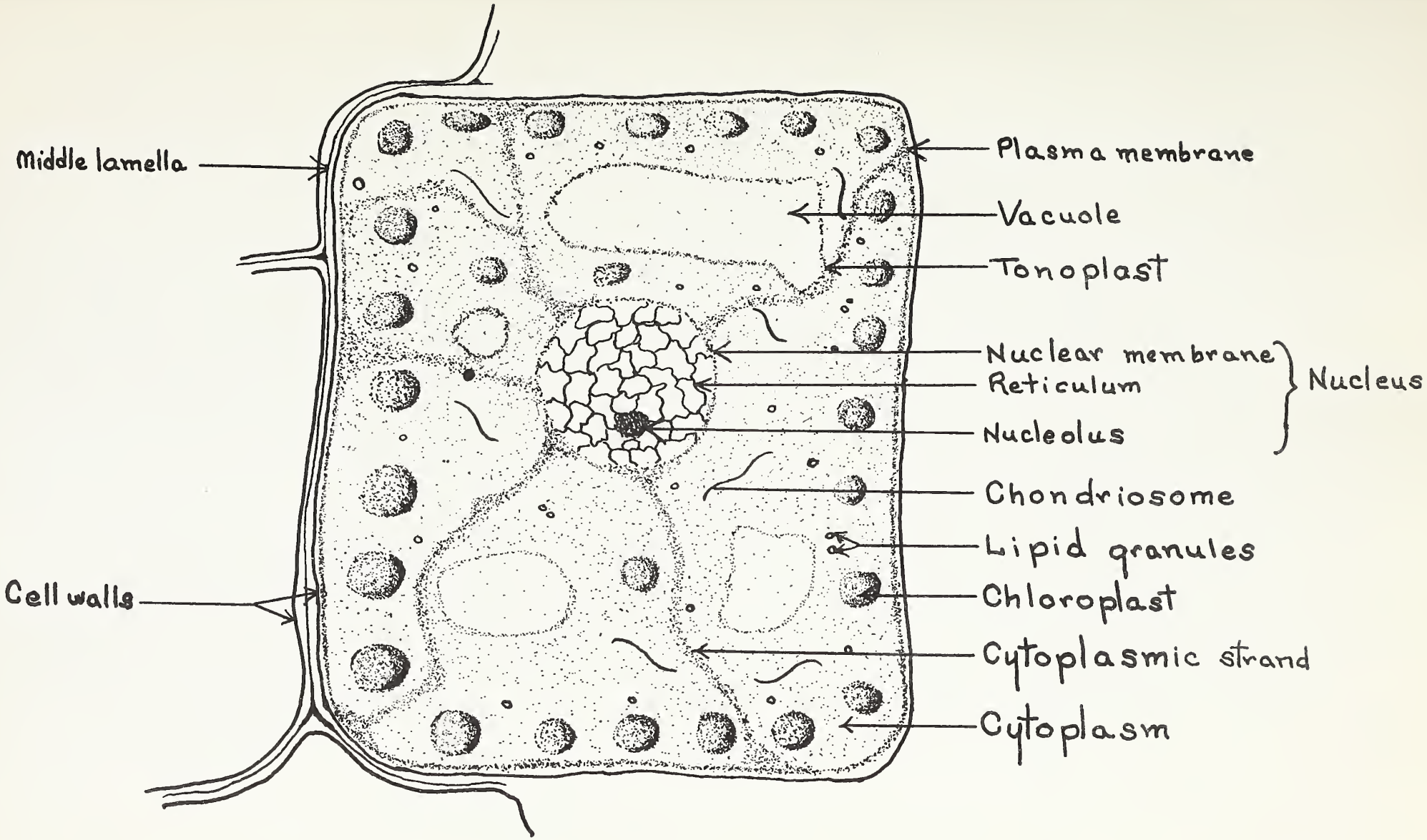
I have included the next slide on vitamins of alfalfa for the sake of completeness in the hope that the proceedings of this conference may serve as a reference for interested persons. This is a good time to point out that all of the analyses on this and the preceding slides may represent some samples of 18% protein alfalfa meal, but the data in most cases are entirely too limited to give even the limits of variability which might be expected. In order to help out along this line, the Western Regional Research Laboratory has planned contract work to determine all water-soluble vitamins in alfalfa meals of varying protein content from the Eastern, Midwestern and the Far Western regions of the country.

The only very extensive data available on variability of alfalfa as a function of location, season, etc., are those on protein and carotene. The next slide shows the average monthly changes in protein and carotene content of lots of alfalfa produced in an Eastern Kansas plant. Of course, all of you producers of dehydrated alfalfa know all about this in your battle to produce a quality product. Affecting the curves are the management of fields with regard to cuttings, the percentages of grass and weeds in the stands, the rainfall, the temperature and undoubtedly many other factors. Nonetheless, the pattern is remarkably constant from year to year. I believe the best generalization which can be made is that the dominant factor which controls quality is the rate of growth of the plant expressed as percent increase per unit of time, rather than as pound per acre increase per unit of time. The next slide shows similar data on alfalfa produced throughout the year at a Louisiana plant. The same type of change occurs.

In looking at the monthly averages shown in the last two slides, one might think that the correlation between carotene and protein might be good enough to predict one by the other. In order to test the accuracy of such predictions, the data from seven successive years were studied statistically. Some of the results are shown in the next slide. It will be noted that the average protein and carotene figures are reasonably constant year by year. Correlation coefficients vary from about .6 to .9, which means that a relatively high degree of correlation does exist. In order to determine how useful this might be in estimating one from an analysis of the other, regression curves were fitted to each year's data and the confidence limits were calculated. The figures in the last column show that if the protein is known, the carotene can be predicted to have a 95% probability of falling within 6 to 8 mg. % of the calculated value. Perhaps this will be clearer from the next slide which shows pooled 7-year data. The line shows the regression curve. If the protein value, for example, is 20%, the carotene read off the curve would be 21.3 mg. % plus or minus 8.16 mg. %. This low precision was somewhat disappointing. Preliminary calculations indicate that using a separate curve for each month greatly improves the prediction since fall production, in general, has a relatively high carotene-protein ratio and therefore tends to weaken the correlation.

My final slide shows the correlation between carotene and xanthophyll content in the samples which were used by Dr. Thornton in his work. Although statistical analyses have not been done on these data, they appear to show a relatively high degree of correlation. Since stabilities of carotene and xanthophylls are quite similar, it can be stated that meal guaranteed to have a high carotene content will also be rich in xanthophylls.

This brings me to the conclusion of my discussion. In closing, I would like to emphasize that, although the information on a few constituents of alfalfa and other forages is quite extensive, we are still a long way from knowing much about the factors which affect the variations in concentration of most of the materials known to be present. What is still more challenging is the fact that we have as yet only scratched the surface on determining the nature and value of the more difficultly measurable, biologically active components such as the grass juice factor, the estrogens, the lactation-stimulating factors, the cellulose-digestion-stimulating factors, the bloat-producing factors, and the growth inhibitors. These are the major problems for research in the future.


Middle lamella
Cell walls
Plasma membrane
Vacuole
Tonoplast
Nuclear membrane
Reticulum
Nucleolus
Nucleus
Chondriosome
Lipid granules
Chloroplast
Cytoplasmic strand
Cytoplasm

A PLANT CELL

VALUABLE FORAGE CROP COMPONENTS

	Ruminants	Non-Ruminants
1. Fat-soluble Vitamins		
Carotene	Yes	Yes
α-tocopherol	Yes	Yes
Vitamin K_1	No	Yes
2. Xanthophylls	No	Yes*
3. Water-soluble Vitamins		
B vitamins	Yes	Yes
Unidentified growth or lactation stimulators (e.g. grass juice factor, estrogen)	Yes	Yes
4. Protein	Yes	No
5. Energy	Yes	No
6. Minerals	Yes	Yes

*Pigmentation in poultry and eggs.

USELESS OR DELETERIOUS COMPONENTS
OF LEGUME FORAGES

	Ruminants	Non-Ruminants
1. Fiber.	--	x
2. Lignin	x	x
3. Bloat-producing factors (e.g. saponin)	x	--
4. Growth inhibitor (saponin).	--	x
5. Estrogen (sterility in ewes). .	x	--

PROXIMATE ANALYSIS OF DEHYDRATED ALFALFA

	%
Crude protein.	18
Crude fiber.	26
Crude fat.	5
Ash.	11
Nitrogen-free extract.	40

COMPONENTS OF THE CRUDE PROTEIN FRACTION
OF 18% ALFALFA MEAL

	% of Total N	% of Alfalfa
True proteins (cytoplasmic . . chloroplast . . enzymes	70	12.6
Free amino acids	18	3.2
Purines and pyrimidines. . . .		+
Stachydrine		.25
Betaine.		.45
Vitamins		+
Chlorophyll.		1.0

ESSENTIAL AMINO ACIDS (FREE + PROTEIN)
IN ALFALFA MEAL (ESTIMATED)

	As % in Crude Protein		
	Alfalfa	Heart Muscle	Wheat
Arginine.	5.0	7.7	3.8
Histidine	1.5	1.7	1.8
Lysine.	5.0	6.9	2.3
Tryptophane	1.3	0.6	1.0
Leucine	10.2	11.0	7.0
Isoleucine.	4.8	3.0	3.2
Phenylalanine . . .	5.0	4.9	4.3
Valine.	6.0	3.6	3.6
Threonine	5.0	3.5	2.8
Methionine.	1.8	1.4	1.6
Cystine	1.9	1.1	1.8
Glycine	4.0	?	?

NON-ESSENTIAL AMINO ACIDS
(FREE + PROTEIN) IN ALFALFA MEAL (ESTIMATED)

	As % Protein
Tyrosine.	4.0
Glutamic acid	10.0
Aspartic acid	8.0
Alanine	5.0
Proline	2.0
Serine.	5.0
Amide ammonia	1.2

COMPONENTS OF THE "CRUDE FIBER" AND "N.F.E." FRACTIONS OF ALFALFA

	% in Alfalfa Meal
A. Structural Components	
Cellulose	30.0
Hemicelluloses	10.0
Lignin	10.0
Ca pectate	6.0
B. Water Soluble	
Sugar (after hydrolysis)	2.8
Starch	1.7
Aconitic acid	.2
Malic, malonic and citric acids	(2.3)
Ascorbic acid	1.0
Succinic acid	.2
Fumaric acid	.2
Saponins	.8
Inositol	.07
Tricin	.02
Coumestrol	Ca. .01

COMPONENTS OF THE "CRUDE FAT" FRACTION OF ALFALFA MEAL (ESTIMATED)

	% in Alfalfa Meal
Triglyceride	2.4
Waxes (hydrocarbon, primary alcohols, esters)	1.2
Sterols (mostly β-sitosterol)	.1
Phosphatide	.03
Calcium phosphatidate	.04
Xanthophylls	.04
α-tocopherol	.02
β-carotenes	.02
Vitamin K_1	.0025
2-hexenol and hexenone	Present
Phytoene	.0004
Phytofluene	.0001

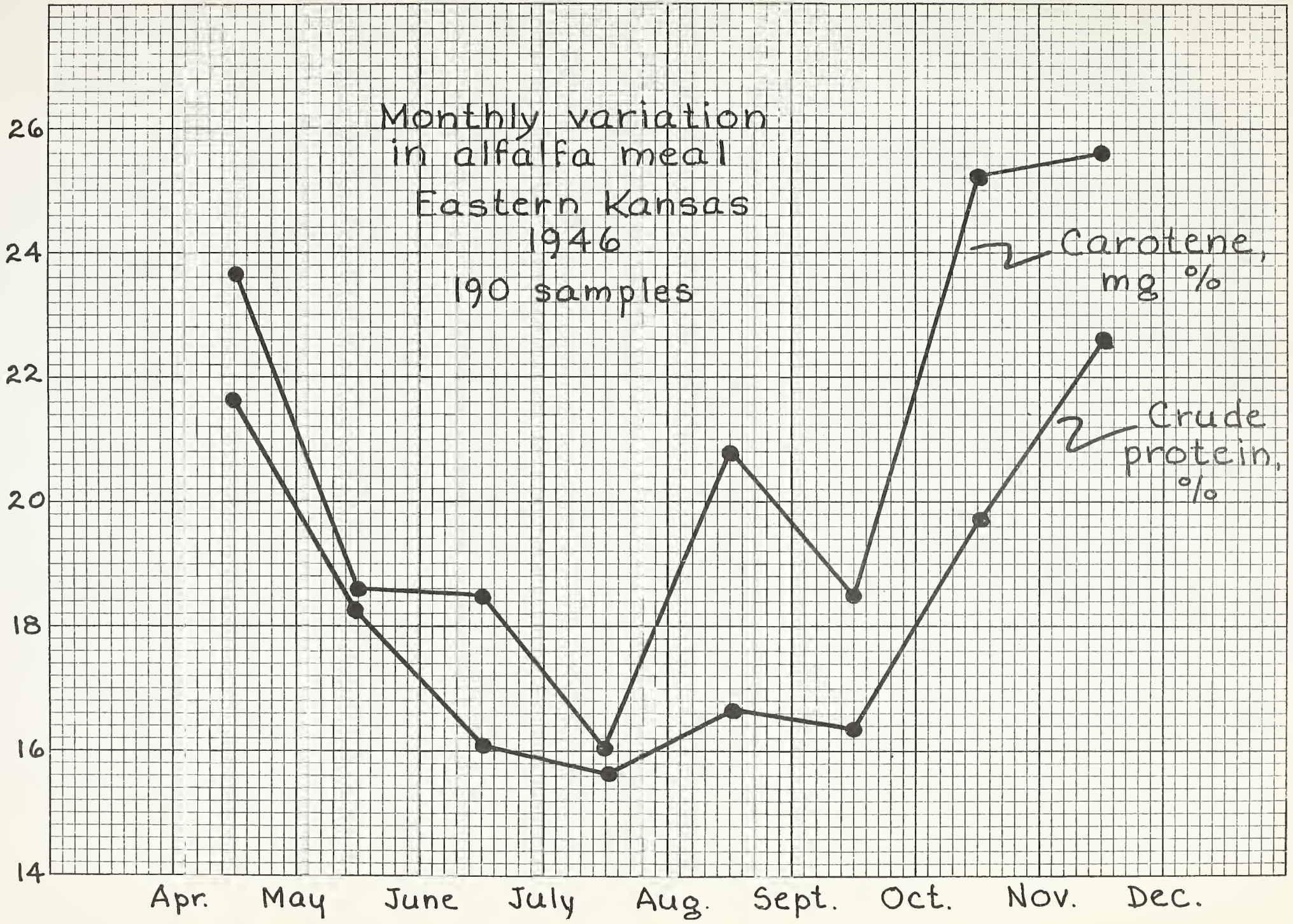
COMPONENTS OF THE "ASH" FRACTION
OF ALFALFA

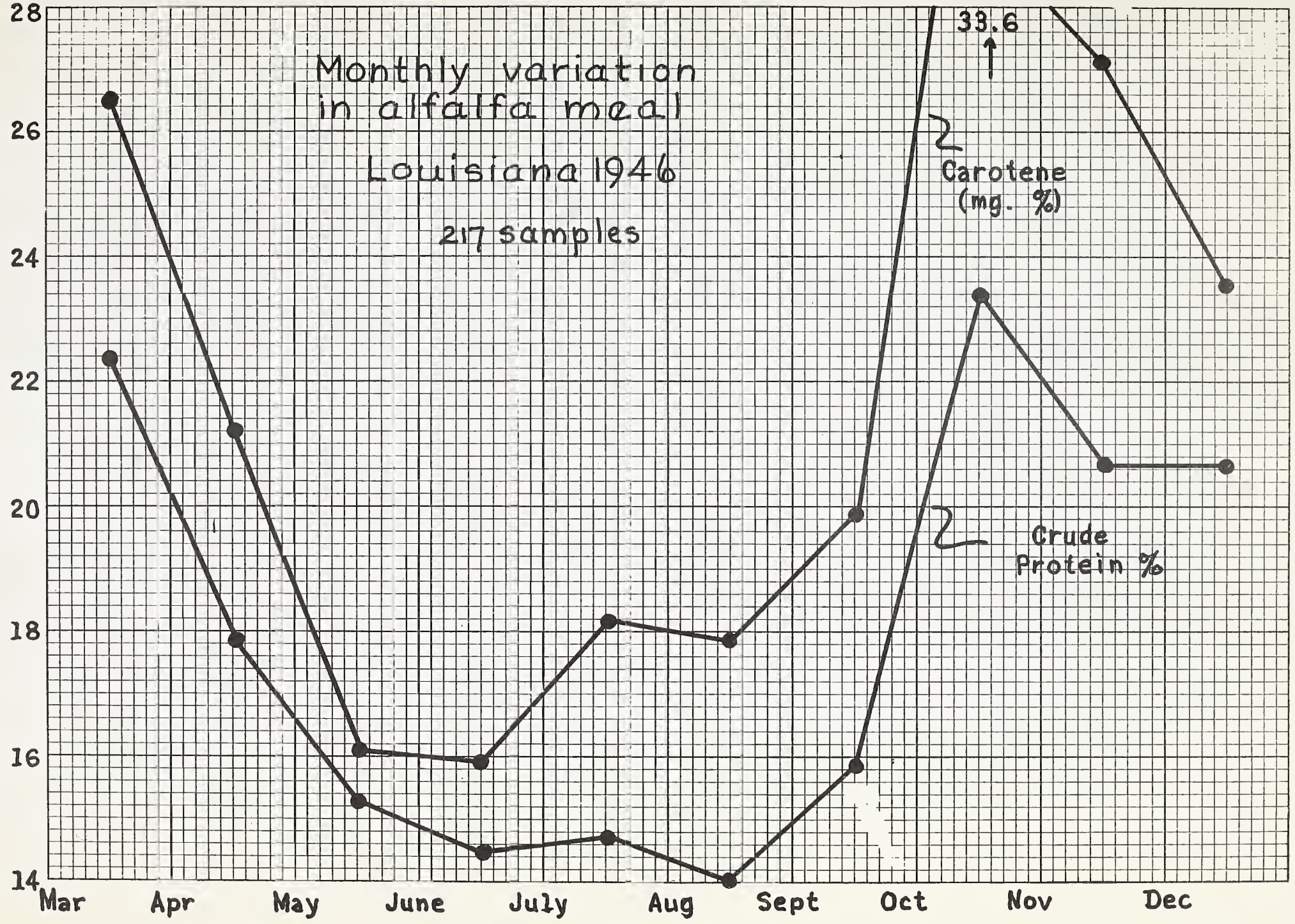
	%
Calcium	1.59
Phosphorus	.31
Magnesium	.31
Potassium	2.6
Sodium	.17
Iron	.09
Manganese	.006
Copper	.004
Cobalt	.00002
Boron	.005
Total	5.09
Total Ash	10.8

KNOWN VITAMINS AND GROWTH FACTORS
IN ALFALFA

	Mg./lb.		Mg./lb.
β-Carotene	90	Niacin	18
Choline	400	Pantothenic acid	16
Ascorbic acid	700	Folic acid	4
Inositol	950	Thiamine	3
Vitamin K	35	Pyridoxine	6
Vitamin E	110	Biotin	150 mcg./lb.
Riboflavin	7	*Thioctic acid	275 mcg./lb.

*Leaf meal

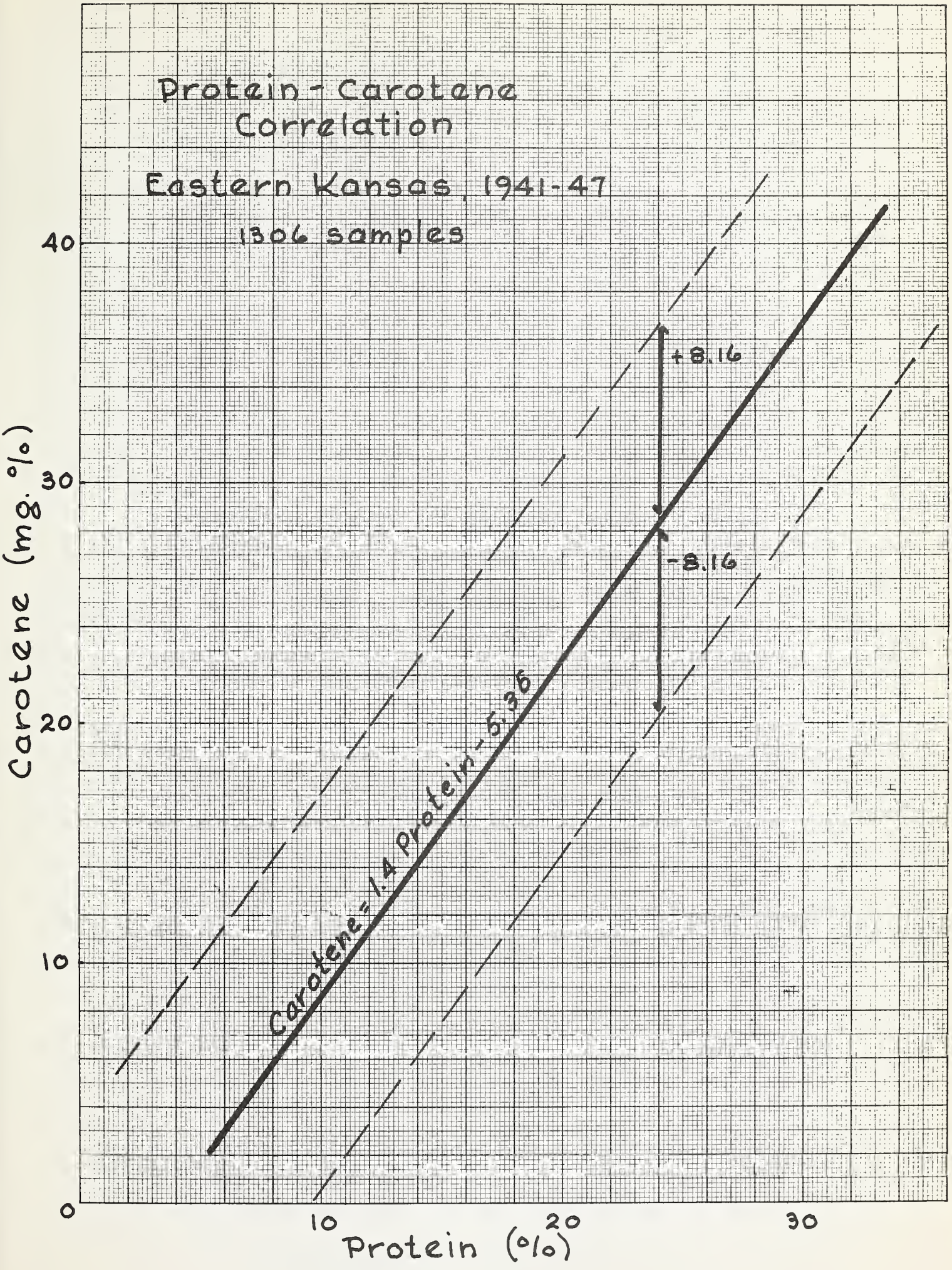

Monthly variation
in alfalfa meal
Eastern Kansas
1946
190 samples
Carotene,
mg %
Crude
protein,
%
26
24
22
20
18
16
14
Apr.
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.

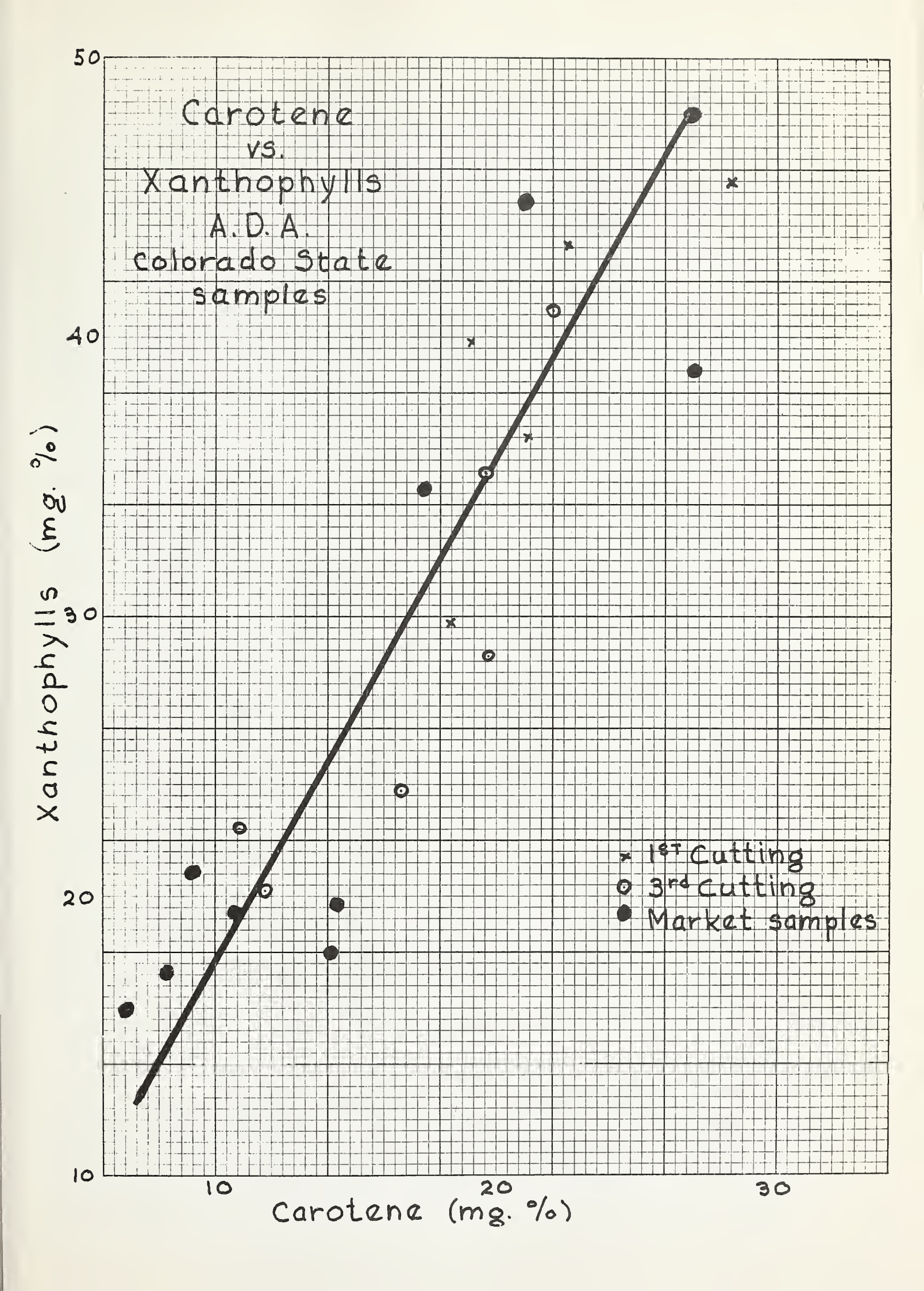

Monthly variation
in alfalfa meal
Louisiana 1946
217 samples
33.6
Carotene
(mg. %)
Crude
Protein %
28
26
24
22
20
18
16
14
Mar
Apr
May
June
July
Aug
Sept
Oct
Nov
Dec

PROTEIN-CAROTENE CORRELATIONS

Eastern Kansas Plant

Year	No. Samples	r.	Avg. Car.	Avg. Prot.	95% Limits on Est. Carotene
1941	147	.710	22.52	18.26	± 9.25
1942	135	.889	19.64	17.58	± 6.31
1943	151	.731	19.33	17.75	± 10.17
1944	109	.644	18.40	18.17	± 8.75
1945	173	.621	19.62	18.54	± 8.11
1946	190	.819	20.03	17.64	± 6.01
1947	401	.840	19.59	17.97	± 7.02
Combined	1306	.757	19.87	17.98	± 8.16


Protein - Carotene
Correlation
Eastern Kansas, 1941-47
1306 samples
+8.16
-8.16
Carotene = 1.4 protein - 5.36
Carotene (mg. %)
Protein (%)
0
10
20
30
40


Carotene
vs.
Xanthophylls
A.D.A.
Colorado State
samples
50
40
30
20
10
Xanthophylls (mg. %)
10
20
30
Carotene (mg. %)
1st Cutting
3rd Cutting
Market samples

OCCURRENCE AND STABILITY OF VITAMIN E, K_1, AND CAROTENE IN DEHYDRATED AND SUNCURED ALFALFA MEAL

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Departments of Chemistry and Poultry Husbandry
Colorado State University
Fort Collins, Colorado

This project originated as a result of a contract between the United States Department of Agriculture and the Colorado Agricultural Experiment Station, a unit associated with the Colorado State University at Fort Collins, Colorado. The task was assigned to the Chemistry and Poultry Sections cooperating. Dr. W. E. Pyke, Chemistry Department Head, was designated as project leader. The responsiblity for the vitamin E and carotene determination was charged to the Chemistry Department; the vitamin K_1 analysis to the Poultry Department.

Dr. C. Ray Thompson of the Feed and Forage Processing Unit of the Field Crops Utilization Section of the Agricultural Research Service, was designated representative of that Unit.

Mr. Joseph Chrisman, executive vice president of the American Dehydrators Association, volunteered to make arrangements necessary to obtain the samples required for the project. This entailed a great amount of work, and we wish to express the thanks of the Chemistry and Poultry Sections to Mr. Chrisman for his efforts and cooperation.

Our thanks also go to the American Dehydrators Association for their cooperation. We are especially grateful to those members who supplied the various samples delivered to us at Fort Collins at their own expense.

Market samples by various industrial firms were also supplied for which we are most appreciative.

Experimental Plan

Six U. S. producing areas were used as a source of samples for the storage experiments, slide 1. Dehydrated alfalfa meals from the first and third cuttings were selected and a suncured sample when available.

Slide 1

These samples, in approximately 100-pound lots, were shipped to Fort Collins by either rail or truck. This slide gives a rather complete history of each sample, particularly pertinent is the column showing the days elapsing from time of cutting to date received. This time ranged from 9 to 67 days. This wide range in transit time resulted from a truck strike which occurred in 1955 and caught some samples in transmit while delaying shipment of others.

This delay may help explain some of the data which are to be discussed later.

Slide 2 shows the contributing industrial firms which supplied the commercial samples. These markets were widely distributed extending from Richford, Vermont to Petaluma, California.

Slide 2

On receipt, all samples were thoroughly mixed and divided into sub-samples for various treatments. These included the initial sample, 12 weeks' storage and 24 weeks' storage samples. The initial samples were placed in a deep freeze at -20° C., which supposedly arrested further deterioration of the vitamins to be tested. The stored samples were maintained at 25° C., in a dark room for 12 and 24 weeks, respectively. At termination of each storage period the sample was then placed in the deep freeze under the conditions previously described.

Other sub-samples were treated with certain antioxidants before storage. These treatments included; Santoquin at 0.015 percent in 1.0 percent cottonseed oil blended initially by hand followed by mechanical mixing for ten minutes and the same treatment as the previous method except the mixed product was wetted completely with Skellysolve and sealed for one hour. The sample was then exposed to air in a dark room at 25° C., until the solvent had evaporated followed by the Santoquin treatment. Other treatments included storage in a sealed container under nitrogen and treatment with 0.015 percent DPPD in one percent cottonseed oil. Storage conditions were the same throughout for all samples.

The commercial samples were not stored. Therefore, determinations were made only for the initial period or date received. These samples were maintained at -20° C., as previously described until complete analysis could be made.

The moisture content of all samples was measured by vacuum oven losses and all values reported in this paper are on a dry matter basis.

Since the carotene and vitamin E analyses were made simultaneously the analytical techniques and results of these two compounds will be discussed first. The carotene and tocopherol levels were determined by a combined procedure worked out in the Chemistry Department of Colorado State University. Carotene was determined colormetrically after chromatographic separation by means of a magnesia Supercell column. Tocopherol was determined on a molecular distillate by means of color development by $FeCl_3$ and α, α-dipyridyl.

Results

The effects of storage on carotene deterioration for the first cutting are shown in slide 3. The carotene content of the initial samples ranged from 9.9 to 31.4 mg./100 grams of dry weight alfalfa. This is approximately

equivalent to 61,000 to 195,000 I.U. of vitamin A activity per pound of dry weight alfalfa. It is interesting to note that sample 501 which was delayed in shipment for a period of 32 days had a particularly low value. Sample 401 was 56 days in arriving; however, the value of this sample was quite high comparatively. It is possible that holding conditions during this period were different between the two samples. Rate of carotene loss with storage appeared to take place at a constant rate. This reaction appeared to be fairly independent of the environment. For example, the loss seemed to be fairly constant within each sample, despite the difference in magnitude of loss between samples.

Slide 3

The effect of storage time on vitamin E deterioration for the first cutting of alfalfa dehydrated meal is shown in slide 4.

Slide 4

In some instances the rate of loss of vitamin E was very similar to carotene loss; however, it appeared that this rate changed after the first 12 weeks and that vitamin E was more stable thereafter. The initial level of vitamin E in sample 501 was approximately equal to other samples. Therefore, the factor believed to reduce the carotene level in this sample did affect the vitamin E level.

The level of carotene and its rate of loss in the third cutting dehydrated meal is shown in slide 5. Sample 203, which has a very low carotene content, was delayed in shipment for 67 days. Again it appeared that this delay may have had something to do with the initial loss of carotene. The rate of carotene loss was again approximately constant.

Slide 5

The vitamin E content, slide 6, for sample 203 group was shown to be very high and its rate of deterioration rather slow. This suggests the possibility that the rapid rate of destruction of carotene may have acted in some way to improve vitamin E stability. Further, it appeared that vitamin E was very stable compared to carotene.

Slide 6

The effect of antioxidants on carotene in storage are shown in slide 7. These results indicated that Santoquin alone was very ineffective as a protector of carotene. The wetting with Skellysolve prior to Santoquin treatment appears to have increased the antioxidant property very readily for a period of 12 weeks and the effect then seems to disappear.

Slide 7

Sealing the material under nitrogen appears to have been a very effective means of preventing carotene breakdown during the 24-week storage period. The effect of antioxidants on vitamin E preservation, slide 8, shows results very similar to those found with the carotene samples. This would indicate that there was a high degree of correlation between the rate of deactivation of these two vitamins, particularly when some method of preservation is employed.

Slide 8

To further illustrate the possibility of such correlation, a graph (slide 9) was constructed which shows the relationship between these two compounds. These results indicated that there was a high degree of correlation between carotene and tocopherol. The coefficients of correlation were approximately 0.91 straight line integration for both the first and third cutting meals. When meals of all types and results were thrown together the coefficient correlation was 0.85. Analysis of variance was determined on these data. These results showed that the tocopherol values were different to a highly significant degree between producing areas and between storage time. There were no significant differences between cuttings for the entire experiment, however, there were highly significant differences within areas between cuttings.

Slide 9

Vitamin K_1 determination was made by the chick assay method. This method is based on the rate of blood coagulation which, in turn, is governed by the amount of vitamin K_1 available in the chick's diet.

The basal ration, slide 10, was given to chicks for a period of seven days. This ration is practically free of vitamin K_1. After seven days this same ration was supplemented with various levels of vitamin K_1 and given to the chicks for the following seven days.

Slide 10

After this period, cardiac punctures were made on each chick and time of blood coagulation or prothrombin time determined. Using these times for each group, a standard curve was constructed, slide 11.

Slide 11

Other groups of chicks were treated similarly, except that after the first 7-day period the alfalfa samples were supplemented into the ration at three different levels. Levels used were 0.1, 0.2, and 0.3 percent of the ration. The prothrombin time of each chick was determined the same day as the standard chicks. Prothrombin times for each individual chick were then fitted to the standard curve and calculated to micrograms of vitamin K_1 per gram of dry weight alfalfa.

White Leghorn cockerels were used throughout these trials. These chicks were all purchased from the Poehlman Hatchery of Petaluma, California. A standard curve was determined for each experiment. The mean $\pm$ the standard error was determined for each group and the "t" test was applied to measure differences.

Loss of vitamin K_1 during storage appeared to be very slight (slide 12) in both first and third cuttings of the dehydrated meals. This loss was of a significant nature in the suncured sample, however. A dehydrated check of this suncured sample also showed a significant loss between 12 and 24 weeks of storage when tested at the 0.1 percent level. You will note in this slide and others that generally the vitamin K_1 values tend to become smaller as the level of alfalfa tested was increased.

At first, this was believed to be due to an excess of vitamin K_1 at the higher level of alfalfa supplementation; however, the same condition exists where alfalfa levels of vitamin K_1 were both high and low. This suggested the possibility that some other factor was present when the alfalfa level was increased.

In lieu of these differences, the discussion of this paper will be limited to values determined at the 0.1 percent level of alfalfa supplementation.

Slide 12

In the second series (slide 13), there were no significant losses of vitamin K_1 during storage of either cutting. However, the Santoquin treated third cutting sample value was lower to a significant degree at 24 weeks. In all dehydrated samples the level of vitamin K_1 dropped markedly between 12 and 24 weeks of storage, however.

Slide 13

There appeared to be no effects from storage either in the absence or presence of Santoquin in the 300 series of samples, slide 14.

Slide 14

In the 400 series (slide 15), vitamin K_1 losses in the first cutting dehydrated meal appeared to follow the carotene pattern. This loss was not prevented by the use of DPPD and was actually greater since the loss was of a highly significant nature after 24 weeks of storage. The vitamin K_1 values also dropped after 12 weeks' storage on the third cutting samples; however, 24-week values were comparable to the initial samples in both cases.

Slide 15

In the 500 series (slide 16), the effect of sealing the samples under nitrogen during storage was tested. The chick assay method did not give a definite pattern here since those samples had values lower than the untreated samples. Values did not change from 12 to 24 weeks, indicating that vitamin K_1 loss did not occur under this treatment.

Slide 16

In the 600 series (slide 17) no losses for the first cutting occurred; however, a highly significant decrease occurred between 0 and 12 of the third cutting sample. A rather marked drop also occurred in the suncured sample which was of a nonsignificant degree.

Slide 17

In slide 18 the vitamin K_1 values for the various commercial samples are shown.

These samples ranged from 28.0 to 39.7 micrograms of vitamin K_1 per gram of dry weight alfalfa. These values are comparable to those of the first and third cuttings of the dehydrated meals just discussed.

Slide 18

Summary

Carotene losses during storage appeared to occur at a constant rate which seemed to be independent of the environment. Treatment with Skellysolve and Santoquin appeared to protect carotene for 12 weeks' storage under the conditions of this experiment. Sealing the meal under nitrogen during storage gave protection for the full 24 weeks.

Vitamin E losses during the first 12 weeks of storage were similar to carotene losses. After this time vitamin E appeared to be more stable. Treatment of alfalfa with antioxidants or sealing under nitrogen protected vitamin E in a manner very similar to carotene.

In a comparison of alfalfa vitamin E and carotene content, under the conditions of this experiment, a high degree of correlation was shown. This suggests the possibility that knowledge concerning the level of one vitamin may be used to predict the level of the other.

Vitamin K_1 stability did not appear to be affected during storage under the conditions mentioned here. There were no consistent differences in vitamin E, K, or carotene content in first and third cutting dehydrated alfalfa meal. Suncured samples contained a much lower level of all three vitamins than that found in dehydrated samples.

Dehydrated Alfalfa Meal - Storage Series Histories

(1955 Truck Strike Caused Delays in Shipping and Delivery)

(Part 1)

Series Numbered	100	200	300
Supplier	Dixon Dryer Co.	Grayson Alf. Dehy. Mills	Rohloff Bros. Inc.
Location	Dixon, Calif.	Grayson, Texas	Graytown, Ohio
First Cutting Date	4-1-55	5-2-55	5-3-55
First Cutting Shipped	4-15-55	5-7-55	5-8-55
First Cutting Received	4-29-55	5-14-55	5-16-55
Days from Cutting Date (Received)	28	12	13
Third Cutting Date	6-14-55	7-7-55	7-18-55
Third Cutting Shipped	6-22-55	8-30-55	7-19-55
Third Cutting Received	7-12-55	9-12-55	7-27-55
Days from Cutting Date (Received)	28	67	9
Days between 1st & 3rd Cuttings	74	65	85
Percent Protein 1st Cutting	20.3	24.7	22.0
Percent Protein 3rd Cutting	23.7	18.3	--
Carotene, 1st Cutting, I.U. per lb.	204,000	235,000	204,000
Carotene, 3rd Cutting, I.U. per lb.	167,700	133,300	--
Irrigated	yes	no	no
Variety of Alfalfa	Buffalo	Southwest Common	Atlantic
Yield in Tons per Acre, 1st Cutting	1.25	0.75	0.95
Yield in Tons per Acre, 3rd Cutting	1	--	1
Condition at 1st Cutting	leafy bud	leafy bud	v. leafy prebud
Condition at 3rd Cutting	leafy prebud	--	stemmy 1/2 bloom
Age of stand	3 years	5 years	3rd year
Soil Type	yolo fine loam	black	Brookstone clay
Fertilization	none	200*,00-20-0	400*, 0-12-12

(Continued)

Dehydrated Alfalfa Meal - Storage Series Histories

(1955 Truck Strike Caused Delays in Shipping and Delivery)

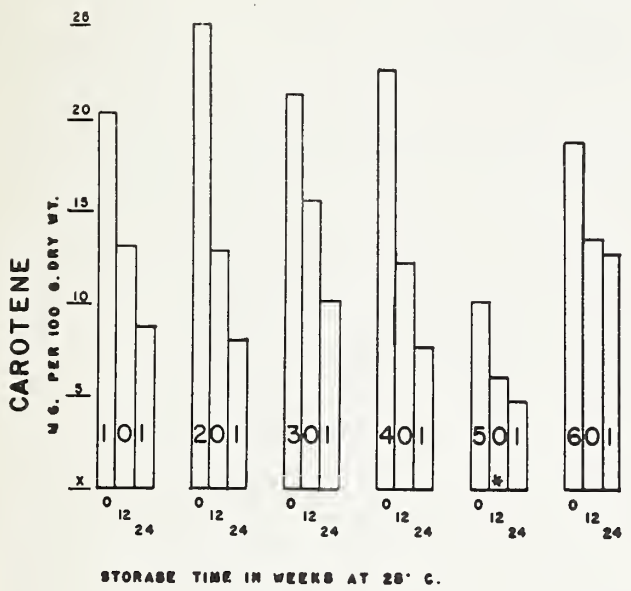
(Part 2)

Series Numbered	400	500	600
Supplier	Neb. Alf. Farms, Inc.	Nat'l. Alf. Dehy. & Mlg. Co.	Hayward, Inc.
Location	Lexington, Neb.	Johnstown, Colo.	El Centro, Calif.
First Cutting Date	4-26-55	6-20-55	11-14-55
First Cutting Shipped	6-2-55	7-22-55	11-17-55
First Cutting Received	6-21-55	7-22-55	11-23-55
Days from Cutting Date (Received)	56	32	9
Third Cutting Date	8-19-55	9-8-55	4-2-56
Third Cutting Shipped	8-27-55	10-21-55	4-9-56
Third Cutting Received	9-2-55	10-21-55	4-21-56
Days from Cutting Date (Received)	14	40	19
Days between 1st & 3rd Cuttings	84	79	138
Percent Protein 1st Cutting	19.3	20.0	--
Percent Protein 3rd Cutting	17.1	19.6	20.9
Carotene, 1st Cutting, I.U. per lb.	177,500	175,000	--
Carotene, 3rd Cutting, I.U. per lb.	131,000	153,000	185,000
Irrigated	yes	yes	yes
Variety of Alfalfa	Ranger Certified	Colorado Common	African, 1st Cut. Calif. Common 21-5, 3rd Cutting
Yield in Tons per Acre, 1st Cutting	1.75	--	0.5
Yield in Tons per Acre, 3rd Cutting	1.50	2	1.25
Condition at 1st Cutting	bud	leafy 1/2 bloom	v. leafy prebud
Condition at 3rd Cutting	Leafy & stemmy full-bloom	--	leafy bud
Age of stand	3 years	3 years	3rd year
Soil type	silt loam	heavy loam	medium
Fertilization	some manure	none	none

SOURCE OF OPEN MARKET SAMPLES

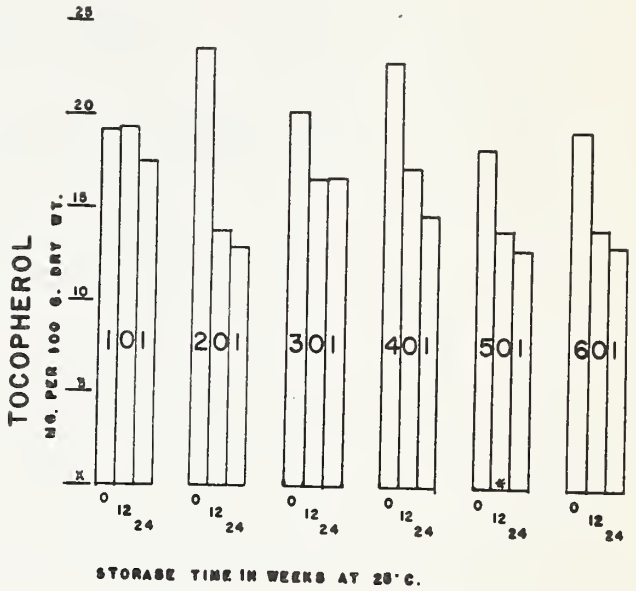
			Number
Chas. M. Cox Co.	Boston, Mass.	Calvert Milling Co. Hoytville, O.	701
Quaker Oats Co.	Cedar Rapids, Ia.	Dawson Co. Feed Products, Lexington, Nebr.	702
Ralston Purina Co.	Ft. Worth, Texas	Bert and Wetta, Gothenburg, Nebr.	703
Uncle Johnny Mills	Houston, Texas	H. E. Co. (Hackney, Ks.) Winfield, Kan.	704
Allied Mills, Inc.	Chicago, Ill.	Allied Mills, Inc. Cozad, Nebr.	705
McMillen Feed Mills	Ft. Wayne, Ind.	Reimer Alfalfa Wakenda, Mo.	706
Poultry Producers of Cent. Calif.	Petaluma, Calif.	Jerry Fielder's Dehydrator Dixon, Calif.	707
H. K. Webster Co.	Lawrence, Mass.	W. J. Small Co., K.C., Mo.	708
H. K. Webster Co.	Richford, Vt.	Saunders Mill, Toledo, O.	709
Poultry Producers of Cent. Calif.	Petaluma, Calif.	Poultry Producers Dehydrator Ryer Is., Calif.	710

DEHYDRATED ALFALFA MEAL
FROM SIX PRODUCING AREAS
CHANGE IN CAROTENE CONTENT
DURING STORAGE AT 25° C.
(FIRST CUTTING)



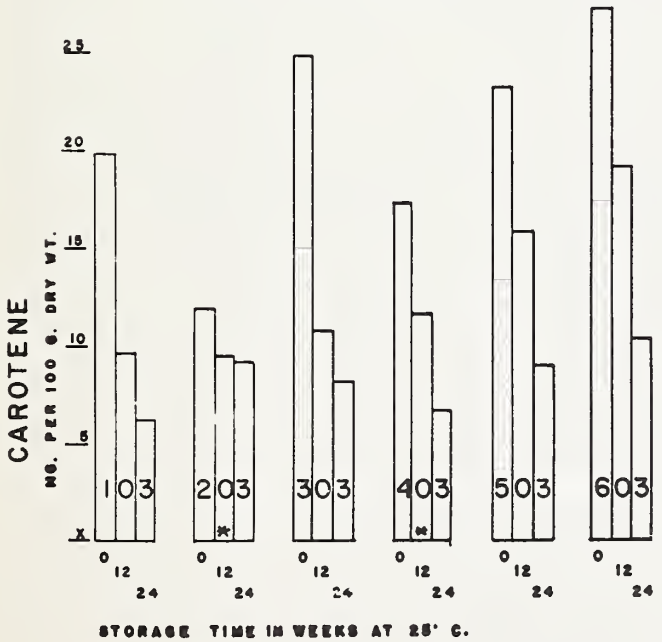
SLIDE 3

DEHYDRATED ALFALFA MEAL
FROM SIX PRODUCING AREAS
CHANGE IN VITAMIN E CONTENT
DURING STORAGE AT 25° C.
(FIRST CUTTING)



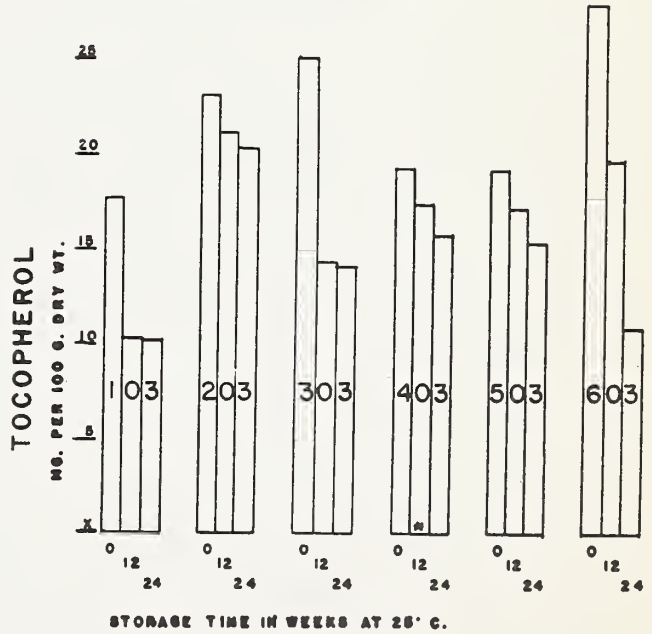
SLIDE 4

DEHYDRATED ALFALFA MEAL
FROM SIX PRODUCING AREAS
CHANGE IN CAROTENE CONTENT
DURING STORAGE AT 25° C.
(THIRD CUTTING)



SLIDE 5

DEHYDRATED ALFALFA MEAL
FROM SIX PRODUCING AREAS
CHANGE IN VITAMIN E CONTENT
DURING STORAGE AT 25° C.
(THIRD CUTTING)



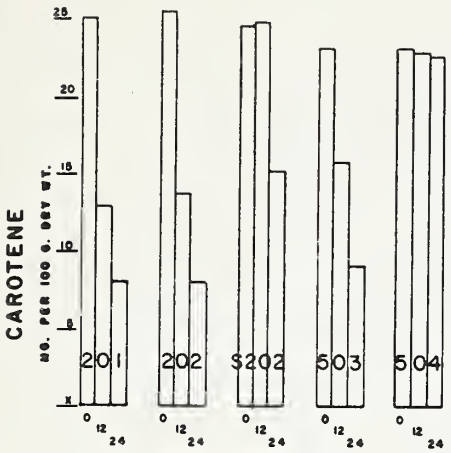
SLIDE 6

COMPOSITION OF THE BASAL RATION

Ingredient	Level (percent)
Sucrose	53.1
Soybean Meal (50%)	41.4
Ground Limestone	0.5
Steamed Bone Meal	2.5
Salt (Iodized)	0.5
Methione	0.5
Corn oil	1.0
$MnSo_4$	0.022
Choline Chloride (70% Aqueous)	0.286
Vitamin Mixture*	0.240

* Vitamin mixture contained the following per kilogram of ration: Vitamin A, 20,000 IU; Vitamin D_3, 4350 ICU; Riboflavin, 10 mg; Niacin, 50 mg; Calcium Pantothenate, 30 mg; Thiamine, 10 mg; Pyridoxine, 10 mg; Folic Acid, 2 mg; Biotin, 0.2 mg; Vitamin B_{12}, 10 micrograms.

DEHYDRATED ALFALFA MEAL
EFFECT OF ANTIOXIDANTS
ON CAROTENE DURING STORAGE



201 – UNTREATED SAMPLE, AS RECEIVED

202 – 201 MIXED WITH 1% COTTONSEED OIL CONTAINING 0.016 % SANTOQUIN

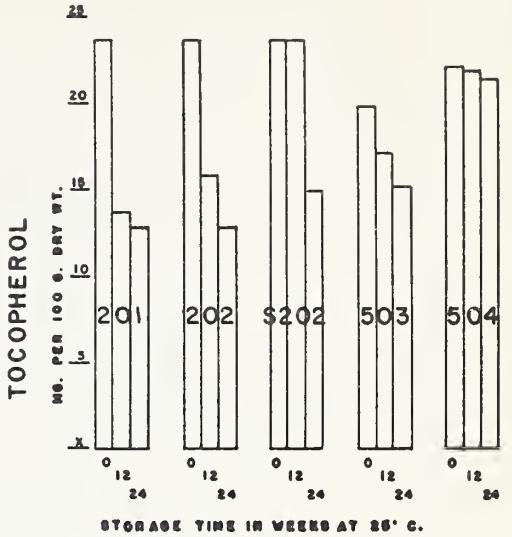
S202 – AS 202 BUT WITH ADDED SKELLYSOLVE TO GET BETTER PENETRATION

503 – UNTREATED SAMPLE, AS RECEIVED

504 – 503 SEALED UNDER NITROGEN

SLIDE 7

DEHYDRATED ALFALFA MEAL
EFFECT OF ANTIOXIDANTS
ON VITAMIN E DURING STORAGE



201 – UNTREATED SAMPLE, AS RECEIVED

202 – 201 MIXED WITH 1% COTTONSEED OIL CONTAINING 0.016 % SANTOQUIN

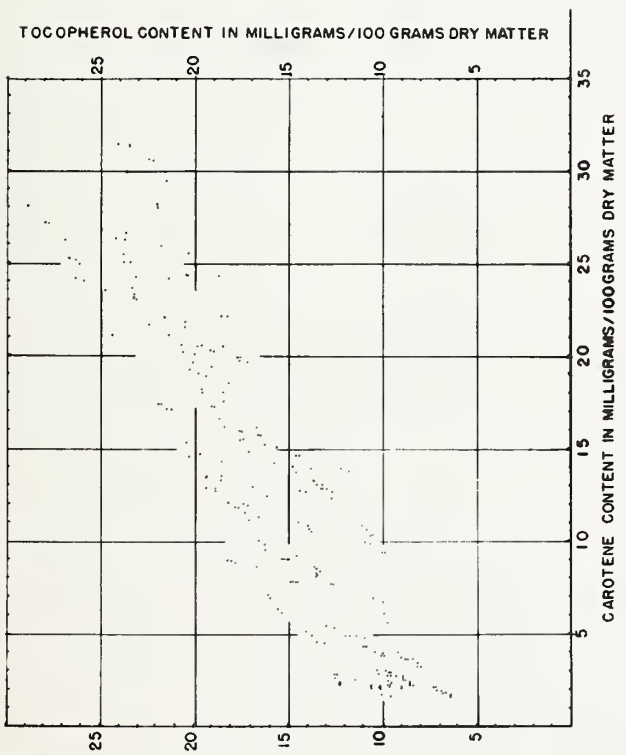
S202 – AS 202 BUT WITH ADDED SKELLYSOLVE TO GET BETTER PENETRATION

503 – UNTREATED SAMPLE, AS RECEIVED

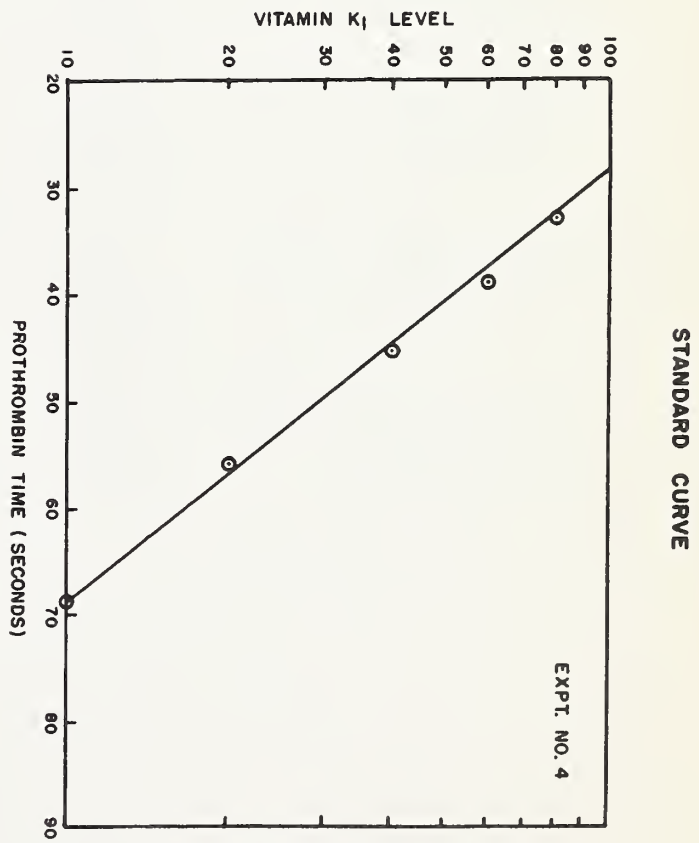
504 – 503 SEALED UNDER NITROGEN

SLIDE 8

CAROTENE VS TOCOPHEROL CONTENT
OF COMMERCIAL ALFALFA MEAL-1955 CROP.



SLIDE 9



STANDARD CURVE

SLIDE 11

STABILITY OF VITAMIN K_1 IN ALFALFA MEAL DURING STORAGE

Sample No.	Treatment	Micrograms Vitamin K_1 per gram alfalfa at levels tested			Cutting	Source and Description
		0.10	0.20	0.30		
101	None	26.6	25.6	26.1	1 st	Dixon, Calif.
101_{S12}	"	27.9	26.3	25.3	"	Dehydrated
101_{S24}	"	26.8	22.9	21.7*	"	
102	Santoquin	25.9	22.7	22.3	1 st	Dixon, Calif.
102_{S12}	in oil	25.1	25.3	24.3	"	Dehydrated
102_{S24}	"	28.4	24.4	22.6	"	
103	None	28.0	28.5	23.7	3 rd	Dixon, Calif.
103_{S12}	"	27.7	26.4	23.6	"	Dehydrated
103_{S24}	"	27.7	26.5	26.0	"	
104	Santoquin	27.1	25.5	21.0	3 rd	Dixon, Calif.
104_{S12}	in oil	25.1	25.7	25.4	"	Dehydrated
104_{S24}	"	27.7	19.7**	23.0	"	
107	None	17.7	13.8	15.2	1 st	Dixon, Calif.
107_{S12}	"	12.8*	12.0	12.1*	"	Suncured
107_{S24}	"	12.3*	12.8	10.4**	"	
108	None	27.9	28.1	24.5	1 st	Dixon, Calif.
108_{S12}	"	24.9	21.1**	22.2	"	Dehydrated
108_{S24}	"	20.1*	20.5**	18.8*	"	Check of 107

* Significant Loss
** Highly Significant Loss

STABILITY OF VITAMIN K_1 IN ALFALFA MEAL DURING STORAGE

Sample No.	Treatment	Micrograms vit K_1 per gram alfalfa at levels tested			Cutting	Source & Description
		0.10	0.20	0.30		
201	None	33.5	30.6	24.9	1st	Sherman, Texas
201_{S12}	"	32.8	31.1	27.9	1st	Dehydrated
201_{S24}	"	26.9	24.3*	25.3	1st	
202	Santoquin	38.7	28.2	26.0	1st	Sherman, Texas
202_{S12}	in oil	38.0	33.9	25.8	1st	Dehydrated
202_{S24}	"	35.8	33.2	26.5	1st	
203	None	40.2	28.6	24.1	3rd	Sherman, Texas
203_{S12}	"	33.7	26.5	26.3	3rd	Dehydrated
203_{S24}	"	32.6	31.1	28.9	3rd	
204	Santoquin	33.4	24.3	24.6	3rd	Sherman, Texas
204_{S12}	in oil	33.0	24.3	24.5	3rd	Dehydrated
204_{S24}	"	25.8	21.2	23.9	3rd	
207	None	18.5	18.2	13.6	1st	Sherman, Texas
207_{S12}	"	14.8	10.5	13.8	1st	Suncured
207_{S24}	"	19.3	15.7	16.7	1st	
208	None	21.4	16.6	15.1	3rd	Sherman, Texas
208_{S12}	"	18.3	13.1	12.6	3rd	Suncured
208_{S24}	"	16.5	13.4	18.1	3rd	

* Significant Loss
** Highly Significant Loss

STABILITY OF VITAMIN K_1 IN ALFALFA MEAL DURING STORAGE

Sample No.	Treatment	Micrograms vit K_1 per gram alfalfa at levels tested 0.10	0.20	0.30	Cutting	Source & Description
301	None	30.9	31.8	24.0	1st	Graytown, Ohio
301_{S12}	"	31.5	24.1**	20.9	1st	Dehydrated
301_{S24}	"	32.0	20.3**	22.1	1st	
302	Santoquin	30.9	27.9	25.9	1st	Graytown, Ohio
302_{S12}	in oil	32.3	27.3	22.6*	1st	Dehydrated
302_{S24}	"	25.3	26.8	22.6*	1st	
303	None	31.7	28.2	27.2	3rd	Graytown, Ohio
303_{S12}	"	30.3	25.7	23.5**	3rd	Dehydrated
303_{S24}	"	34.4	27.8	25.2	3rd	
304	Santoquin	35.5	30.5	25.6	3rd	Graytown, Ohio
304_{S12}	in oil	32.9	26.3	22.1	3rd	Dehydrated
304_{S24}	"	31.8	34.2	24.6	3rd	
307	None	17.4	14.4	12.0	1st	Graytown, Ohio
307_{S12}	"	18.4	9.8*	9.4	1st	Suncured
307_{S24}	"	20.8	9.0*	9.2	1st	

* Significant Loss
** Highly Significant Loss

STABILITY OF VITAMIN K_1 IN ALFALFA MEAL DURING STORAGE

Sample No.	Treatment	Micrograms Vitamin K_1 per gram alfalfa at levels tested			Cutting	Source and Description
		0.10	0.20	0.30		
401	None	40.7	27.4	23.0	1 st	Lexington, Nebr.
401_{S12}	"	35.8	26.1	20.6	"	Dehydrated
401_{S24}	"	32.9	18.4**	22.7	"	
402	DPPD	38.1	26.0	22.6	1 st	Lexington, Nebr.
402_{S12}	in	29.6	20.1	23.8	"	Dehydrated
402_{S24}	oil	24.4**	23.5	18.9*	"	
403	None	38.3	24.1	23.4	3 rd	Lexington, Nebr.
403_{S12}	"	28.9	24.0	18.4	"	Dehydrated
403_{S24}	"	37.1	25.4	21.6	"	
404	DPPD	33.0	26.7	20.7	3 rd	Lexington, Nebr.
404_{S12}	in	27.1	22.8	20.9	"	Dehydrated
404_{S24}	oil	33.3	26.4	18.7	"	
407	None	21.6	19.4	14.7	1 st	Lexington, Nebr.
407_{S12}	"	20.0	15.3	16.0	"	Suncured
407_{S24}	"	21.6	18.9	14.3	"	

* Significant Loss
** Highly Significant Loss

STABILITY OF VITAMIN K_1 IN ALFALFA MEAL DURING STORAGE

Sample No.	Treatment	Micrograms Vitamin K_1 per gram alfalfa at levels tested			Cutting	Source and Description
		0.10	0.20	0.30		
501	None	34.8	25.7	20.3	1 st	Johnston, Colo.
501_{S12}	"	32.4	25.8	25.5	"	Dehydrated
501_{S24}	"	30.4	22.3	20.2	"	
502_{S12}	Sealed	27.8	23.4	23.4	1 st	Johnston, Colo.
502_{S24}	Under N_2	29.3	23.1	21.4	"	Dehydrated
503	None	37.7	23.6	23.7	3 rd	Johnston, Colo.
503_{S12}	"	42.2	29.5	27.9	"	Dehydrated
503_{S24}	"	33.9	25.9	26.4	"	
504_{S12}	Sealed	27.7	22.1	21.3	3 rd	Johnston, Colo.
504_{S24}	Under N_2	28.4	26.7	22.0	"	Dehydrated
507	None	15.4	12.8	16.7	1 st	Johnston, Colo.
507_{S12}	"	17.6	16.8	14.8	"	Suncured
507_{S24}	"	22.4	14.7	11.3**	"	
508	None	32.2	30.7	23.6	3 rd	Johnston, Colo.
508_{S12}	"	29.2	20.8	19.8	"	Suncured
508_{S24}	"	31.0	32.4	27.5	"	

* Significant Loss
** Highly Significant Loss

STABILITY OF VITAMIN K_1 IN ALFALFA MEAL DURING STORAGE

Sample No.	Treatment	Micrograms vit K_1 per gram alfalfa at levels tested 0.10	0.20	0.30	Cutting	Source and Description
601	None	32.0	26.7	23.3	1st	Heber, California
601_{S12}	"	33.7	20.3	18.5	1st	Dehydrated
601_{S24}	"	33.6	30.8	28.0	1st	
603	None	38.2	29.9	24.7	3rd	Heber, California
603_{S12}	"	26.5**	27.7	25.7	3rd	Dehydrated
603_{S24}	"	27.6**	28.6	23.5	3rd	
607	None	25.0	17.0	17.1	1st	Heber, California
607_{S12}	"	20.2	13.6	9.3**	1st	Suncured
607_{S24}	"	20.4	10.8**	9.9**	1st	

* Significant Loss
** Highly Significant Loss

STABILITY OF VITAMIN K_1 IN ALFALFA MEAL
DURING STORAGE

Sample No.	Micrograms vit K_1 per gram alfalfa at levels tested			Source (Market Source)
	0.10	0.20	0.30	
701	36.2	25.9	22.8	Boston, Mass.
702	28.0	25.4	19.5	Cedar Rapids, Iowa
703	29.7	22.4	19.2	Fort Worth, Texas
704	30.0	22.7	22.9	Houston, Texas
705	39.7	30.3	23.2	Chicago, Ill.
706	29.3	25.8	21.4	Fort Wayne, Ind.
707	35.6	31.4	26.2	Petaluma, Calif.
708	35.5	24.6	25.5	Lawrence, Mass.
709	36.9	26.4	24.7	Richford, Vt.
710	29.8	26.1	27.6	Petaluma, Calif.

OCCURRENCE AND NATURE OF ESTROGENS IN GREEN FORAGE CROPS

E. M. Bickoff

Western Regional Research Laboratory
Albany, California

Within recent years the relationship of estrogens to animal nutrition has received widespread attention. The efficiency of meat production can be increased and the composition of the meat can be improved by hormone administration. On the other hand, when animals graze on forage containing excessive amounts of estrogen-like materials, serious reproductive disorders may result, with consequent decrease in fertility, stillbirth, or early death of the young. Estrogens may thus have beneficial as well as adverse effects on animals and it becomes important that we learn as much as we can about their occurrence in animal feeds and forage.

Before discussing the main topic of this talk, I should like to say a little about the natural estrogens that occur in the animal body, and their function. Next, I should like to discuss the synthetic or artificial estrogens. Probably the hormone that has received the most attention in this respect is diethylstilbestrol. After that, I plan to briefly mention the nature of the estrogens that have been isolated to date from plants other than green forages, and then finally discuss the work that has been done to date on green forage crops, with perhaps a suggestion for possible further work that may well be done along these lines.

Natural Animal Estrogens

In the female animal, the follicular hormone or estrogen is produced by the ovaries. Its production marks the beginning of ovulation and the estrus cycle. Removal of the ovaries stops sex development. The secondary sexual characteristics do not appear, or if present, tend to disappear. A reverse effect may be induced by administering ovarian extracts or estrogens. Administration of estrogen to the male animal prior to maturity inhibits the normal development of the male sex characteristics and promotes the development of female sex characteristics.

The natural estrogens that have been found to occur in animals are all chemically related derivatives of cyclopentanophenanthrene having the same basic structure but differing in the number and position of substituents. Estrone is not the most active of the estrogenic substances but is the standard of comparison for the activity of related compounds. Estrone is much more effective after injection than by oral administration. Estradiol is about 4 to 8 times as effective as estrone. Estriol is only about 1/10 as active but its action is somewhat more protracted. (Equilenin is 1/10 and Equilin 1/3 as active as estrone.)

Synthetic or Artificial Estrogens

This lack of molecular specificity in the series of natural estrogens led Dodds and his colleagues about 1930 to examine how the molecule of a natural estrogen might be changed without destroying estrogenic activity. This work culminated in 1938 in the discovery of the estrogenically potent synthetic compounds diethylstilbestrol, hexestrol and dienestrol. These compounds remain the most potent, useful synthetic estrogens. Their discovery had immediate importance in estrogen therapy since previously, only natural products had been available and their administration necessitated injection. The synthetic compounds were found to possess all of the qualitative estrogenic properties of the natural estrogens, were cheap to produce and were active by mouth. They have attained considerable clinical importance.

Estrogens for Poultry

One of the earliest agricultural uses for stilbestrol was in the chemical "castration" or caponizing of cockerels. The first suggestion that birds might be fattened for market by estrogen administration was made by Prof. F. W. Lorenz, of the Department of Poultry Husbandry, University of California at Davis, in 1943, with data on the effects of subcutaneous implantation of diethylstilbestrol pellets in cockerels.

Along with the change in secondary sex characteristics, administration of estrogen to poultry causes an increase in the rate of fat deposition as well as causing additional effects that might enhance quality or improve the economy of rearing birds for the market. Thus, when the cockerels were implanted with small amounts of stilbestrol, the growth rate and feed efficiency of the birds were increased. In addition, there was a definite improvement in the tenderness and desirability of the flesh of the birds treated in this manner.

Stilbestrol implants continue to be used rather widely in the poultry industry, both in chickens and in turkeys.

Estrogens for Ruminants

A great deal of interest has developed over the period of the past three years regarding the use of stilbestrol in the feeding of growing-fattening cattle, based on the continuing favorable response from its use. Burroughs and co-workers at Iowa State College, reported as early as 1954 and 1955 that the feeding of five to 10 milligrams of stilbestrol per animal daily resulted in an increase of about 20% in the rate of gain, reduced the feed requirements and increased the daily feed consumption.

There continues to be considerable interest in the use of hormones to stimulate the rate and efficiency of gains in steers. In the interval since the early reports by the Iowa researchers, many papers have been published showing the benefits derived from the oral administration of stilbestrol in the rations of fattening cattle.

The Food and Drug Administration has approved the use of implants of stilbestrol within the past year.

Estrogens in Plants Other than Forages

The occurrence in plants of substances capable of causing estrus in animals was first reported in 1926. Since then, many plant extracts have been examined, and a large number of these have been reported to show some estrogenic activity. Unfortunately, very few of the positive results obtained in preliminary tests on plants have been followed by larger scale examination and isolation of the plant estrogen.

Estrogenic constituents have now been isolated from several plants and it is clear that although these constituents are in some cases identical with normal animal estrogens, this identity is by no means universal.

Palm Kernels: The first isolation and characterization of an estrogen from a plant source was accomplished in 1933 from the residual cake left on pressing palm kernels. The estrogen was shown to be identical with estrone, which is one of the most active of the natural estrogenic substances, and which had originally been isolated from the urine of pregnant mares. This work was of considerable interest and importance in that it demonstrated that a sex hormone normally secreted and required by animals was also a normal constituent of certain plant products.

Willow catkins: Closely following this isolation of estrone from palm kernels, there was a report of the isolation of an estrogen from willow flowers. This product closely resembled estriol, another of the sex hormones, also originally isolated from pregnant animal urine. In 1947, the pollen grains of the date palm were found to have a product closely resembling estrone.

Butea superba: Schoeller in 1940 obtained from the tubers of the Siamese vine, Butea superba, an estrogen of very great activity which is definitely different from any known member of the follicular hormone group obtained from animals. Its empirical formula was given as $C_{19}H_{22}O_6$ whereas the follicular hormone, estrone, has the formula $C_{18}H_{22}O_2$. According to this author, this hormone is about 20 or 30 times as effective as the natural follicular hormone when taken by mouth.

The isolation of this estrogen marks an important advance in our knowledge of plant estrogens, as it was the first demonstration of the presence in a plant of a highly potent substance, not identical with the normal steroid estrogens of the animal kingdom. It is most unfortunate that investigation of this extremely interesting compound appears to have been discontinued; its structural formula is still unknown, and there is no indication whether it belongs to any known class of plant products or not. It is sincerely to be hoped that further study of its constitution will be undertaken in the near future.

Other Plants

Similarly estrogen concentrates have been obtained by Costello and Lynn from licorice root, but they have not been able to obtain any pure compound showing estrogenic activity.

Estrogens in Forage Crops

In 1941 there appeared with spectacular suddenness an outbreak of infertility in sheep in a large part of Western Australia. The areas affected, in all over 8000 square miles, were all of low rainfall and in all of them the predominant pasture was subterranean clover. Since this clover had been the predominant pasture for 15 years, the sudden outbreak was difficult to explain on the basis that the clover was responsible, but this proved to be the case. A combination of wartime factors (e.g. shortage of fertilizer and of bulk feed) and climatic conditions had caused a much greater intake of clover per sheep for a long period.

The symptoms included failure of ewes to conceive, stillbirth or early death of lambs, and various disorders of the female reproductive system. Rams were not affected. The lambing percentage sometimes dropped to as low as 8%, and transfer of ewes to good non-clover grazing areas for three successive seasons did not restore fertility. The only measures which proved effective were based on reduction of the amount of clover grazed, and on the observation that the clover was most potent in early spring.

Early attempts to isolate the substance responsible were handicapped by the lack of suitable methods of assay. This problem was finally solved in 1948 by Curnow, Robinson, and Underwood, who measured the increase in uterine weight of ovariectomized mice when fed on a diet containing the dried ether-soluble material from a known amount of clover. Robinson subsequently improved this method by injecting ether-soluble material into immature whole mice. In our Pharmacology laboratory, Dr. Booth feeds the estrogen concentrate mixed in the normal diet of the animal.

By feeding increasing amounts of pure estrogen to mice, a graded series of responses will be obtained from which a dosage-response curve may be obtained.

In the same way by feeding or injecting increasing amounts of the forage being tested for estrogenic activity, a dosage-response curve may be constructed.

Using this technique to guide them, Bradbury and White finally isolated from about 9000 lbs. of clover, the estrogens which were responsible for the reproductive problem in sheep. Genistein was shown to be the principal estrogen and was about 10^{-5} times as active as stilbestrol.

Genistin had earlier been shown to be a constituent of soybean meal by Dr. Edmund Walter, a member of our Feed and Forage Processing Unit.

Genistin is the glycoside of genistein.

Genistin activity on a weight basis was slightly lower than that of genistein. The two compounds appeared to have approximately equal activity on a molecular basis.

A related estrogen, daidzein has also been found as a glycoside in soybean meal but not in forages.

Subsequently, formononetin and biochanin A have also been shown to be constituents of subterranean clover and all three of these estrogens have also been found in Red Clover.

Although no direct evidence is yet available in regard to the value of forage estrogens for promoting increased rate of growth of livestock a recently reported experiment by Professor Loyal C. Payne of Iowa State College may indirectly bear on this point. At the Iowa Station, in two separate lamb feeding experiments they were unable to confirm the growth-stimulating effect of stilbestrol. At the end of the feeding period, there were no noticeable differences between the control and the stilbestrol treated lambs. It was thought possible that the clover hay on which the lambs were feeding might have been contributing estrogen. Estrogen assay of the clover hay yielded results which appeared to be sufficiently high enough to elicit an estrogenic response if we can assume that the clover estrogen acts similarly to stilbestrol. Needless to say, the significance of estrogenic substances found in feedstuffs commonly fed to farm animals merits further study.

In our laboratory, we have recently succeeded in isolating an estrogen from ladino clover in crystalline form. This material is different in structure from any of the known animal estrogens. It is also different from the isoflavones previously isolated from subterranean clover and red clover. Its structure which is still under investigation does not appear to be that of any previously reported compound, either isolated from plant sources or prepared synthetically. The compound is about 30 times as potent as

genistein, the most potent estrogen previously reported in forage crops, although it is considerably less potent than diethylstilbestrol. The quantity of estrogen present in a plant may vary greatly at different times.

Professor Andrews of Purdue University Experiment Station has recently conducted an interesting study of the variation in estrogen concentration that occurs in the alfalfa plant. He found that there was a significant increase in estrogen in the early budding stage, followed by a decline until fourth bloom and during the seed head stage. The pattern of estrogenic activity in terms of stage of plant growth and total quantity during the second, third and fourth crops was considerably different than that of the original spring growth.

The second cutting did not show the large increase during early budding and did not increase significantly until the dough stage. In both the third and fourth cuttings, the potency of the samples was considerably less than in the spring growth.

In studying the distribution of estrogenic activity in the alfalfa plant, it was found that alfalfa leaves were more active than flowers and the stems had the least activity.

Dr. Andrews also compared estrogen content of alfalfa silage and freshly cut alfalfa. He found that alfalfa silage prepared with blackstrap molasses contained appreciable estrogen--much more than comparable alfalfa in pasture.

In silage that had been stored for approximately 24 months his data suggest that the estrogen activity is greater at the bottom of the silo than at the top.

His data further suggests that the molasses ensiled material produces more estrogenic material than does silage stored without a preservative.

In summary, it appears that there is considerable variation in estrogenic activity between and within plant species and that differences in season, stage of growth and other environmental factors may affect hormonal activity. In addition, that the estrogenic content of the forage may be further increased by preparation of silages.

General Summary

It is quite obvious that our knowledge of plant estrogens is as yet limited and fragmentary. Clearly a great deal more systematic investigation is required on plant extracts to select the more promising sources of estrogens,

followed by the isolation and characterization of the estrogens themselves, before we are able to generalize about their occurrence in various plant families or about their nature.

The present position can be summarized by saying that the known plant estrogens are estrone, estriol, genistein and its derivatives, the Butea estrogen as yet unidentified, and a new and different type of compound discovered at this laboratory and found to be present in at least three different forage materials including alfalfa, ladino clover and strawberry clover.

The active compounds in some 40 other plants, reported estrogenic, have not yet been isolated or characterized and it is highly probable that many other plants contain estrogenic compounds. They should provide a fertile field for future investigation.

REVIEW OF SAPONINS IN FORAGES

C. Ray Thompson

Western Regional Research Laboratory,
Albany, California

Some years ago it was observed by a number of investigators (Colorado, Oregon, and other Experiment Stations) that feeding some alfalfa meals to young chicks at high levels, i.e., 10-20% of the total diet, caused a reduction in growth. Excessive fiber and low energy were blamed, but when these were equalized the birds still did more poorly on the alfalfa meal diets. Thus the presence of a "growth inhibiting factor" in alfalfa was suggested. Studies at University of California by Drs. Peterson and Lepkovsky showed that a fraction isolated from alfalfa meal when fed to birds at a level equivalent to 20% of alfalfa meal in the diet caused reduced growth. This fraction had certain characteristic properties of saponins and it was postulated that saponin was responsible for causing growth inhibition. This finding has been confirmed by Heywang and Bird, who fed more purified preparations of alfalfa saponin from the Western Regional Research Laboratory than Peterson had available. To date no one has answered the question, "Are saponins the only growth inhibitors?" It should be noted that Peterson showed that feeding cholesterol and some other saponin complexing agents overcame the growth inhibition. However, from a practical standpoint no one has been able to show growth inhibition with 5% of alfalfa meal. Ordinarily this gives stimulation, and it is often difficult to show inhibition at much higher levels, so in presently formulated broiler rations the amount of inhibitor in the 1-3% of alfalfa meal used can be ignored.

Another more surprising biological effect has been observed with saponins. Dr. W. D. Maclay suggested that saponin be fed to ruminants. This was done by Lindahl and Ellis, who observed definite symptoms of bloat. Because ruminant bloat causes economic losses in the United States estimated at $40 million annually, we immediately began preparing saponins for further testing. We also devised a rough quantitative method for measurement of saponins in forages.

These studies showed that forages which cause bloat such as alfalfa and clovers are higher in saponins than forages that do not cause bloat. Trefoil, a non-bloating forage, has little saponin and grasses seem to be devoid of these compounds. Alfalfa was found to contain saponins which yielded about one dozen compounds, presumably sapogenins, upon hydrolysis. A paper chromatographic method was developed for separating the different sapogenins. This method also serves for partially separating saponins but will require much additional work before a complete resolution is effected.

Ladino clover showed at least three saponins which upon hydrolysis gave soyasapogenols A, B, and C. These are all triterpenoids. Dr. Carl Djerassi and co-workers at Wayne State University aided in the identification of these three compounds. These sapogenins were also found in alfalfa. Dr. Djerassi's group also characterized the most abundant sapogenin which we were able to isolate from alfalfa. This proved to be a heretofore unrecognized triterpenoid which had two carboxyl and two hydroxyl groups. This was named "medicagenic acid". Another less-abundant sapogenin, tentatively named "lucernic acid", was isolated from alfalfa. This compound has a molecular formula of $C_{30}H_{46}O_7$ and was three hydroxyl groups, one carboxyl group and a lactone structure. Further work is necessary to determine its exact structure. Hederin, a saponin previously isolated from English ivy (Helix hedera), was recognized as a major saponin component of bur clover.

Physiological studies on these compounds have shown that mixed alfalfa saponin will kill fish in dilute solution, hemolyze red blood cells and when added to a solution in which rabbit intestine is immersed will inhibit peristaltic activity and cause rapid relaxation of the strip. Bur clover saponin caused increased tonus but also stopped peristalsis. Saponin from ladino clover caused somewhat similar effects but low solubility of these compounds in water interfered with the tests to some extent.

In cooperative studies with the Department of Animal Husbandry, University of Arizona, we agreed to test samples of alfalfa for saponin that were being fed as green-chopped material to steers at the Yuma Station. Correlation of total saponin content and bloating potential was exceedingly erratic.

However, when paper chromatograms of the sapogenin fractions were prepared and stained, good apparent correlation was obtained between bloating potential and three or four red staining sapogenins which appear at Rf 0.35 - 0.75. Further studies seem to confirm this observation, i.e. bloating samples are rich in these components while non-bloating samples have little.

A comparison of saponins in different parts of alfalfa plants (aerial portion), alfalfa roots and alfalfa seed showed the roots to have blue staining sapogenins almost exclusively, while alfalfa seed was rich in the red staining components and had little of the blues. The aerial portion had some of all of these compounds. Present efforts are directed toward obtaining partially purified saponins which will yield red staining sapogenins for biological testing.

The chemical nature of these compounds is still unknown. We assume them to be saponins because of their physical and chemical behavior but it is necessary that we isolate the parent compounds of these red-staining materials and actually determine their nature.

Physiological tests with saponins prepared by this Laboratory at Cornell University by Dr. R. W. Dougherty have shown that saponins, when introduced into intestinal (duodenal) fistulas, inhibit part of the eructation or belching mechanism of sheep. Dr. Dougherty feels that if intact saponins are carried beyond the rumen in normal digestion that they could account for at least part of the bloat syndrome.

In other studies at the University of Minnesota addition of alfalfa saponin to bovine erythrocytes seems to activate acetylcholine esterase The possible significance of this finding has yet to be defined.

Attendance List

	Name	Organization	Address
1.	Ralph M. Arms	Kern County Land Company	Bakersfield, California
2.	H. F. Beckerdite	Nutrilite Products, Inc.	Buena Park, California
3.	Ralph Beermann	Beermann Bros. Dehydrating Company	Dakota City, Nebraska
4.	Ralph Booze	Western Feed and Seed	San Francisco, California
5.	J. H. Bridges	Kern County Land Company	Bakersfield, California
6.	V. C. Britton	V. C. Britton Company	Firebaugh, California
7.	D. A. Bunting	Nutrilite Products, Inc.	Buena Park, California
8.	L. E. Card	Agricultural Experiment Sta. University of Illinois	Urbana, Illinois
9.	Charles P. Castle	Madera Milling Company	Madera, California
10.	Joseph Chrisman	American Dehydrators Assn.	Kansas City, Missouri
11.	Dale C. Covey	Nutrilite Products, Inc.	Hemet, California
12.	John Crain	Western Feed and Seed	San Francisco, Calif.
13.	Herbert Dalton	California Farm Bureau	Berkeley, California
14.	J. J. Dillard	Nebraska Alfalfa Farms, Inc.	Lexington, Nebraska
15.	W. A. Donnelly	National Alfalfa Dehydration and Milling Company	Lawrence, Kansas
16.	W. B. Dye	Agricultural Experiment Sta. University of Nevada	Reno, Nevada
17.	Roy C. Elrod	Archer-Daniels-Midland Co. (W. J. Small Company)	Minneapolis, Minnesota
18.	Loyd M. Faris	" " " "	Kansas City, Missouri
19.	Jerry W. Fielder	Dixon Dryer Company	Dixon, California
20.	J. B. Finley	Union Feed Yards (Livestock Res. Adv. Com.)	Blythe, California
21.	Glenn Finney	Biological Control University of California	Albany, California

	Name	Organization	Address
22.	Alan G. Forbes	California State Department of Agriculture	Sacramento, California
23.	R. G. Fowler	Farm Journal	San Francisco, Calif.
24.	J. G. Gilmore	California Hay, Grain and Feed Dealers Association	Sacramento, California
25.	H. O. Graumann	Crops Research Division, ARS	Beltsville, Maryland
26.	K. W. Hagen	U. S. Rabbit Experiment Sta.	Fontana, California
27.	H. C. Harvey	Fred De Hoff Company	San Francisco, Calif.
28.	D. J. Hayes	V. C. Britton Company	Firebaugh, California
29.	Roy Hitchcock	California Farmer and Feedstuffs	San Francisco, Calif.
30.	D. B. Hodel	Prairie Dehydrating Company	Roanoke, Illinois
31.	J. K. Holloway	University of California (Entomology Research Division - ARS)	Albany, California
32.	E. A. Hollowell	Crops Research Division, ARS	Beltsville, Maryland
33.	W. L. Howe	Entomology Research Division - ARS	Bakersfield, California
34.	Eunice Hunt	American Dehydrators Assn.	Kansas City, Missouri
35.	R. P. Johnson	Elk Valley Alfalfa Mills	Independence, Kansas
36.	L. G. Jones	University of California	Davis, California
37.	F. H. Kratzer	University of California	Davis, California
38.	Stewart Lockwood	California State Department of Agriculture	Sacramento, California
39.	Bert F. Maxwell	Poultry Producers of Central California	Petaluma, California
40.	S. Atwood McKeehan	Farmer	Meridian, California
41.	Frank Melchoir	Poultry Producers of Central California	San Francisco, Calif.
42.	J. B. Merryfield	Abilene Alfalfa Mill	Abilene, Kansas
43.	J. H. Meyer	University of California	Davis, California
44.	Kenneth Morrison	Morrison & Quirk	Hastings, Nebraska

	Name	Organization	Address
45.	H. A. Pacini	University of Nevada	Reno, Nevada
46.	Robert Pearl	University of California	Davis, California
47.	D. W. Peterson	University of California	Davis, California
48.	J. F. Simonet	Madera Milling Company	Madera, California
49.	R. F. Smith	University of California	Berkeley, California
50.	E. H. Stanford	Department of Agronomy University of California	Davis, California
51.	V. M. Stern	University of California	Riverside, California
52.	G. H. Stockbridge	Kern County Land Company	Bakersfield, California
53.	Orion O. Tatro	Dixon Dryer Company	Dixon, California
54.	R. F. Taylor	" " "	Dixon, California
55.	Stefan Tenkoff	Nutrilite Products Company	Buena Park, California
56.	L. N. Thompson	Poultry Producers of Central California	San Francisco, Calif.
57.	P. A. Thornton	Colorado State University	Fort Collins, Colorado
58.	G. N. Tucker	California Cattle Feeders Association	Los Angeles, California
59.	R. Van den Bosch	University of California	Riverside, California
60.	W. C. Weir	University of California	Davis, California
61.	M. L. Wilson	Agricultural Experiment Sta.	State College, N. M.
62.	K. B. Yetter	California Farmer	San Francisco, Calif.
63.	E. A. Mengering	Farm Bureau Cooperative Assn.	Columbus, Ohio

Personnel from Western Regional Research Laboratory

W. B. Van Arsdel	A. N. Booth	R. L. Lyman
G. O. Kohler	R. H. Wilson	E. D. Walter
K. W. Taylor	V. F. Kaufman	E. M. Bickoff
J. E. Simpson	C. R. Thompson	J. Guggolz
C. H. Kunsman	G. R. Van Atta	A. L. Livingston
F. DeEds	H. P. Binger	A. Paitis